CHAPTER 1. General Introduction

Copper, Fe, Mn and Zn are trace minerals that are essential for broiler growth. They are involved in a large number of digestive, physiological and biosynthetic processes within the body of broiler chickens. Despite the fact that enormous advances have been made in poultry production and technology, research in trace mineral nutrition has lagged behind other areas of nutrition.

Currently, meat chickens can attain a body weight of 2 kg in 35 days, down from 64 days in 1979. However, the trace mineral requirements of broilers are still thought to be at the same level as those recommended by the National Research Council (NRC) for Animal Nutrition (NRC, 1994) more than a decade ago, which, in turn, are based on data from as far back as the 1950s. Typically these levels relate only to inorganic sources of minerals and have as their objective, the avoidance of sub-clinical levels of deficiency. Therefore, it is questionable if these levels are realistic and appropriate for achieving the maximum growth potential of modern birds.

On the other hand, in commercial practice the industry is still using a large safety margin in feed formulation to guarantee the bioavailability of trace minerals. These supplemental inorganic trace minerals result in a high level of mineral excretion. It was demonstrated that the poultry manure applied on a nitrogen basis contained zinc and copper 660 and 560%, respectively, in excess of the crop requirements (Dozier *et al.*, 2003). Due to the concern of build-up of heavy metals when applying poultry litter to cropland, the environmental protection agencies around the world have pressed for lower levels of mineral waste to be applied to land. Europe is also considering imposing constraints on diet formulation. It has been proposed that the total amount of dietary Zn and Cu for broilers should not exceed 40 and 8 mg/kg (including amounts from natural ingredients), respectively (Dozier *et al.*, 2003).

Considering that it is uncertain if performance is optimized by current inorganic supplementation practices, reduction of inorganic supplementation may not be productive (Bruerton, 2005). It is critical to find new sources of trace minerals with

higher bioavailability to replace current inorganic salts in order to limit mineral excretion, but not compromise broiler growth.

In the past 10 years, nutritionists have observed that many trace minerals occur in nature in an organic form and plants have the ability to take up inorganic minerals from the soil and turn them into complexes. Organic complexes such as chelates and proteinates have been shown to be better absorbed and utilized than their inorganic salts (Scott *et al.*, 1982; Leeson, 2003). Based mainly on trace mineral tissue accumulation, organic trace minerals have shown advantage or at least are as efficacious as inorganic salts. However, application of organic trace minerals in animal nutrition, especially in broiler production, is still a new research area. There is a lack of knowledge of the actual organic trace mineral requirements for the modern broiler and limited understanding in regards to organic mineral sources on mineral absorption and excretion. Most reports involving the use of organic minerals for broilers are based on conventional diets, usually in excess of requirements of trace minerals for broilers, as the control diets, leading to lack of growth responses (Henry *et al.*, 1989; Hess *et al.*, 2001; Lee *et al.*, 2001; Paik, 2001; Chowdhury *et al.*, 2004; Revy *et al.*, 2004).

However, growth response in broiler chickens has been used as the primary criterion for determining bioavailability of several trace minerals because the broiler is an ideal assay animal with a limited nutrient storage, high nutrient demand and rapid growth rate (Ammerman, 1995b). Traditionally, the requirements of inorganic trace elements, especially Zn, were determined by purified diets in order to observe the growth response. One of the difficulties often encountered in trace mineral research is that maximum growth is not obtained because the purified diets are poorly consumed (Dewar, 1986). The results derived from purified diets may not be applicable in practical production. It is reasonable to find some natural ingredients containing lower levels of trace minerals, to incorporate into a control diet and allow the birds to reach their genetic potential when required trace minerals are supplemented. Based on the bird growth response, it is possible to determine an optimal amount of organic trace minerals, especially Zn, for broiler chickens in order to set the trace mineral quantities in diets close to requirements, and to minimize waste in the interests of productivity and environment (Thompson and Fowler, 1990).

Interactions among minerals are a major cause of variation in availability, which influence the nutritive value of a diet. It is reported that Zn deficiency strongly depresses animal growth due mainly to reduction in feed intake (King et al., 2000; Shay and Mangian, 2000). When applying organic Zn in broiler production, it is crucial to use an optimal Zn level that would allow birds to reach their genetic potential of feed intake and therefore optimal body weight gain. It is necessary to identify which mineral is the first limiting element and to investigate possible interrelationships among Cu, Fe, Mn and Zn and further, to define molecular mechanisms involved in feed intake regulation, to guide the application of these products in broiler chicken production. There are several peptides that are suggested to be involved in the regulation of feed intake and cholestokinin (CCK) may be a substance involved in feed intake regulation in association with zinc deficiency.

Copper, Fe, Mn and Zn are present within chicken tissues in very small and constant amounts but play an important role in chicken growth. They function primarily as catalysts in enzyme systems within cells or as parts of enzymes and are also constituents of thousands of proteins involved in intermediary metabolism, hormone secretion pathways and immune defence mechanisms (Dieck et al., 2003). They affect animal tissues and cells to such a large extent at only minute amounts that it is difficult to find a suitable biomarker to investigate the status of these minerals and their roles properly at normal supplemental levels.

For example, correlations between the loss of enzyme activity and characteristics of Zn deficiency have been proven unsuccessful and changes in Zn metalloenzymes are not considered the first major causative factor in Zn deficiency that leads to growth depression (MacDonald, 2000). Zinc and growth hormone are considered to act independently in regulation of growth and development (Freake et al., 2001). The immune response of chicks to Zn deficiency is less clear and immune suppression response to varying Zn is not as sensitive as decreased growth responses (Pimentel et al., 1991a). Considering that organic Zn has the potential to be absorbed better and it may have a different metabolic pathway to the inorganic salts, it is necessary to investigate absorption sites and excretion for these organic trace minerals and define Zn molecular mechanisms to guide the application of these products in broiler chicken production.

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Based on a control diet mainly containing sorghum and isolated soy in which the contents of Cu, Fe, Mn and Zn are less than the broiler requirements recommended by NRC but other nutrients meet the specifications of a practical broiler diet, this thesis investigated the efficacy of organic trace minerals, namely Cu, Fe, Mn and Zn, in broiler diets. The key areas of the study were:

- Broiler performance, mineral excretion and accumulation in tissues;
- Levels of supplementation on performance and trace mineral digestibility along the small intestine;
- The optimal supplemental levels for growth and feed efficiency;
- The interactions between Zn and other trace minerals, and
- Mechanism(s) by which Zn regulates feed intake.

CHAPTER 2. Literature Review

2.1 Introduction

The extent of research conducted on different minerals is mainly based on their economic value and dietary deficiency in practical diets (NRC, 1994). Thus there is a great deal of literature concerning the Ca and P requirements of broilers, whereas research into trace mineral nutrition has lagged behind other areas of nutrition due to the low cost of inorganic trace minerals, such as Cu, Fe, Mn and Zn, used in premixes and complete diets of broilers. Although these trace minerals are involved in a large number of digestive, physiological and biosynthetic processes within the body and play an important role in body growth, the requirements of these trace minerals for practical broiler chicken diets are not well defined and only tentative values are given after 3 weeks of age.

Currently, meat chickens can attain a body weight of 2 kg in 35 days and half of this body weight is obtained from 21 d of age. Thus reported requirements of these trace minerals recommended by the National Research Council for Animal Nutrition in the early 1990s (NRC, 1994), which are based on data from as far back as the 1950s, may not be reasonable owing to difference in genetic growth potential and use of ingredients that interfere with the utilization of these trace minerals.

On the other hand, the low cost of inorganic trace minerals and intensive farming have pushed the broiler industry to use large safety margin in feed formulation to guarantee the bioavailability of trace minerals. It is considered that the quantities of minerals to meet the requirement of the animal are affected by their availability in the diets and by other dietary constituents that may affect their absorption or metabolism. In commercial practice. these supplemental inorganic trace minerals result in a high level of mineral excretion. High rates of excretion result in slurry and manure that pollute drainage water or affect plant physiology. For example, under normal commercial dietary conditions, 94% of the Zn ingested is excreted (Mohanna and Nys, 1999). It was demonstrated that the poultry manure decreased the yield of bush bean from 876 to 33 kg/ha when birds were fed a diet containing 360 mg Zn/kg instead of a diet containing 90 mg Zn/kg (Giordano *et al.*, 1975). The poultry manure applied on a nitrogen basis contains Zn and Cu 660% and 560%, respectively,

in excess of crop requirements (Dozier *et al.*, 2003). It is therefore timely to examine how and why these trace minerals are fed to broilers. Is it possible to reduce dietary trace mineral concentrations or improve their bioavailability so as not to compromise broiler growth? Further, there is the issue of dealing with the variation of trace mineral levels in commonly used ingredients, which exists widely even amongst raw materials drawn from the same sources. Furthermore, certain trace minerals can interact with each other and these interactions can cause their bioavailability to vary substantially. Therefore, the trace mineral nutrition is urgently in need of re-visiting.

The organic trace minerals appear to provide a pathway to minimize the trace mineral levels in the excreta and meet the broiler chicken requirements for optimal growth and health. However, application of organic supplements in animal nutrition, especially in broiler chicken production, is a new research area. Due to the complexity of assessment for bioavailabilty of trace minerals, there is a gap between actual organic trace mineral requirements by the modern broiler and supplemental levels of these trace minerals. The exact mechanisms of organic mineral absorption and excretion are not fully understood. There are not enough data to determine optimal levels of supplementation for broiler chickens in practical use.

This review will summarize the literature on current state of trace mineral nutrition and the effect of organically complexed trace minerals (mainly Cu, Fe, Mn and Zn) on broiler production and identify gaps in knowledge of inorganic and organic trace mineral nutrition for broiler birds.

2.2 Overview of mineral nutrition

In addition to C, H, N, O and S, which are the major elements comprising the organic chemical compounds of the body, the chicken requires at least 14 inorganic elements for proper nutrition (Scott *et al.*, 1982). They are often divided into two categories, based on the amount that is required by the bird and present in the diet. The macro elements, usually stated as a percentage of the diet, are important constituents of tissues, playing structural and metabolic roles. The trace minerals are present in small amounts in animal tissues and function primarily as enzyme catalysts and physiological regulators (Thompson and Fowler, 1990).

2.2.1 Macro-minerals

For poultry essential macro-minerals include Ca, P, K, Na and Cl. Calcium and P are essential for the formation and maintenance of the skeleton. Ninety-nine percent of Ca in the diet of broilers is used for bone formation while 80% of the available P is in the skeleton and the remaining 20% is required in the utilization of energy and in structural components of cells (NRC, 1994). Phosphorus in the form of phytic acid is poorly utilized and phytic acid also reduces Ca bioavailability. Therefore, the practical diets fed to broilers are always deficient in Ca and P unless supplemented with inorganic salts. An excess of dietary Ca interferes with the availability of P, Mg, Mn and Zn. A ratio of approximately 2 Ca to 1 non-phytate P is recommended for broilers (NRC, 1994). In growing chicks, optimum body growth and calcification of the bones occur with calcium levels ranging from 0.6% to 1.2%, with an available P level of 0.5% (Scott et al., 1982).

Potassium, Na, Cl are important determinants of acid-base balance (NRC, 1994). Due to the widespread practice by poultry producers of ignoring the contribution of sodium and chlorine from dietary ingredients and drinking water and addition of supplemental salt to diets, there is little concern about the occurrence of practical Na and Cl deficiency (Henry, 1995). The minimum Na and Cl requirements are 0.13% during the starting period and 0.07% during the period after 6 weeks of age. The practical K requirement has been set at 0.4% of the diet. The K content of normal practical diets is usually about 1.0%, well meeting the requirements of the chicken (Scott et al., 1982). Diets containing significant levels of animal by-products will be lower in K, although deficiency is not an issue.

2.2.2 Trace minerals

The common essential trace minerals include Mg, Mn, Zn, Fe, Cu, Mo, Se, I and Co. A number of elements, including fluorine, nickel, silicon, tin, vanadium and chromium, have been shown to produce beneficial effects in chicken nutrition, but have not yet been classified (Scott et al., 1982).

Magnesium is essential for chickens and more than one hundred enzymes have been found to be activated by this element. Normally 500 mg Mg per kg diet is adequate

for all stages of growth and more than 1% Mg in the diet of hens causes marked impairment of performance (Scott et al., 1982). The Mg content in practical poultry diets always exceeds chicken requirements. Therefore, it is not necessary to supplement Mg.

Molybdenum is required for the formation and maintenance of normal levels of xanthine oxidases in the liver and intestine, but the growth retardation due to Mo deficiency is not caused by interference with the activity of this enzyme (Scott et al., 1982). Molybdenum in isolated soybean protein is unavailable to the chick and normally a supplementation level of 0.5 mg Mo per kg diet may be required.

Selenium is an integral component of glutathione peroxidase and it has been found that Se and vitamin E prevent exudative diathesis. Selenium is an essential nutrient for the body's antioxidant system (Choct et al., 2004). The NRC recommended that the Se requirements for broilers is 0.15 mg/kg diet and the toxic levels of Se (10-20 ppm) are approximately 100-fold higher than their requirements (Scott et al., 1982).

Vitamin B_{12} contains 4.4% of Co and dietary Co deficiency in ruminants prevents synthesis of sufficient Vitamin B_{12} by rumen micro-organisms and therefore results in Vitamin B_{12} deficiency. Co deficiency has never been clearly demonstrated in poultry while Vitamin B_{12} is supplemented in broiler chicken diets (Underwood and Suttle, 1999a). Cobalt is also supplemented in the current premix for broiler chickens.

Iodine is required for the synthesis of thyroid hormones and the most obvious consequence of a deficiency is goitre (Mateos et al., 2005). Thyroid hormones control the rate of energy metabolism or level of oxidation of all cells and also affect other endocrine glands. The NRC recommended that I requirement is 0.35 mg/kg diet.

Copper, Fe, Mn and Zn function primarily as catalysts in enzyme systems within cells or as parts of enzymes. In particular, Zn is constituents of thousands of proteins involved in intermediary metabolism, hormone secretion pathways and immune defence mechanisms (Dieck et al., 2003).

2.3 Functions of trace minerals in poultry

Trace minerals are present within chicken tissues in very small and constant amounts but play an important role in chicken growth. The Cu, Fe, Mn and Zn composition of adult and newly hatched chickens is shown in Table 2.1. These trace minerals function primarily as catalysts in enzyme systems within cells or as parts of enzymes. The roles that they play in enzymatic reactions range from weak, ionic strength effects to highly specific associations known as metalloenzymes (Underwood, 1977). They are also constituents of thousands of proteins involved in intermediary metabolism, hormone secretion pathways and immune defence mechanisms (Dieck et al., 2003). They significantly affect animal tissues even in amounts so minute that it is difficult to find a suitable biomarker to investigate the status of these trace minerals and their roles properly. However, a relatively constant ratio of each mineral in the whole body concentration at different age of chickens implies that only total trace mineral contents will increase with the body weight gains. Deficiency of these trace minerals will definitely affect broiler growth and surplus trace minerals will not benefit bird growth and possibly be excreted

Table 2.1 Cu, Fe, Mn and Zn contents of chickens (including feathers) at different ages (mg/kg live weight)

Constituent	At hatch	7 weeks
Cu	1.22	1.28
Fe	34.00	27.20
Mn	0.27	0.18
Zn	14.40	15.40

After Mohanna and Nys (1998)

2.3.1 Enzyme and protein systems

Copper is mainly combined with the protein, ceruloplasmin and it is also found in a large number of metalloenzymes such as cytochrome oxidase, superoxide dismutase, lysyl oxidase, dopamine hydroxylase and tyrosinase (Larbier, 1992). However, ceruloplasmin mRNA in liver has been shown to be unaffected by a dietary deficiency of Cu (Chesters, 1992). Overall, this metal is involved in cellular respiration, cardiac function, bone formation, connective tissue development, keratinization and

pigmentation of tissue, as well as myelination of the spinal cord (McDowell, 1992). Cu has a direct effect on Fe metabolism and thus indirectly affects haemoglobin biosynthesis.

Iron plays an active part in oxidation/reduction reactions and in electron transport associated with cellular respiration. Practically all of the Fe in the animal's body is organic in nature and only a very small percentage is found as free inorganic ions (Henry and Miller, 1995). Two-thirds of body Fe is found as part of haemoglobin, which is involved in oxygen transport and control of cellular respiration. The remainder is distributed among several proteins acting as Fe reserve (Larbier, 1992). In general terms, Fe is essential to cellular and whole body energy, and protein metabolism, and is vital for good health and prevention of anaemia (Kaim and Schwederski, 1993). It has been suggested that this pool performs several productive roles, including supply of iron for the synthesis of iron-containing enzymes, functioning in the process of cellular iron transport and storage, and contributing to the expression or repression of iron-responsive genes (Woodmansee and Imlay, 2002).

Manganese, like other essential trace elements can function both as an enzyme activator and as a constituent of metalloenzymes involved in glycolysation of proteins. Although the number of Mn-metalloenzymes is limited, a large number of enzymes can be activated by Mn. These include hydrolases, kinases, decarboxylases and transferases. Manganese is a vital element for correct bone growth in broilers, for carbohydrate and lipid metabolism, immune and nervous system function and reproduction (Power, 2003).

Zinc is one of the most important trace elements and plays three major biological roles in the body: as a catalyst, and as a structural and regulatory ion (Stefanidou et al., 2006). Zinc is a component of over 300 enzymes in different species of all phyla (Vallee, 1993). These include carbonic anhydrase, alcohol dehydrogenase and alkaline phosphatase. In its association with enzymes, Zn plays an active catalytic role. Zn deficiency could decrease feed intake and slow the growth rate of broilers (Larbier, 1992). However, correlations between loss of enzyme activity and characteristics of Zn deficiency have proven unsuccessful and changes in Zn metalloenzymes are not considered the first major causative factor in Zn deficiency that leads to growth

depression (MacDonald, 2000). Zn–containing enzymes have an extremely high affinity for their metal ligand and thus may retain Zn even in the face of low concentrations (Chesters, 1992).

Apart from this catalytic role in enzyme systems, Zn is also a component of more than 1000 transcription proteins, suggesting that alterations in mineral status will definitely be translated into changes in gene expression (Dieck *et al.*, 2003; Ho *et al.*, 2003). Zinc is also a known inhibitor of enzymes in general (Maret *et al.*, 1999).

2.3.2 Bone growth

The bone is a complex, heterogeneous tissue that supports the musculature and, thus, its growth and development are intimately connected with overall body growth (Loveridge, 1992). Copper, Fe, Mn and Zn are required for the growth, development and maintenance of healthy bone (Beattie and Avenell, 1992).

Copper and Fe deficiency inhibits bone growth and decreases bone strength even when Ca and P levels are sufficient (Medeiros *et al.*, 1997). Copper intake is the main determinant of bone strength, while an adverse effect of increased dietary Zn on bone strength has only been reported with inadequate dietary Ca (Roughead and Lukaski, 2003). This is ascribed to a Cu-containing enzyme, lysyl oxidase, which is responsible for enhancing bone strength (Ilich and Kerstetter, 2000). On the other hand, an overload of Cu and Fe might act as a toxin to bone cell, but there is no clear evidence of this in broiler nutrition.

Manganese is a co-factor for glycosyltransferases, which catalyse the transfer of a sugar from nucleotide-diphosphate sugar to an acceptor molecule (Leach, 1971). Manganese deficiency could lead to thickened long bones and "perosis", the latter being characterized by gross enlargement and malformation of the tibiometatarsal joint, twisting and bending of the distal end of the tibia and thickening and shortening of the tibia bones (Scott *et al.*, 1982).

Zinc is a co-factor of several enzymes for bone mineralization. In broiler chickens, deficiency of Zn could induce shortening and thickening of tibia bones (Scott *et al.*,

1982). Many of the effects of Zn deficiency on bone metabolism may be related to generalized impairment of nucleic acid and protein metabolism (Beattie and Avenell, 1992). However, currently there is no direct evidence.

2.3.3 Hormone metabolism

Growth hormone (GH) plays a key role in growth stimulation but its association with trace mineral deficiency is still not clear. Zinc deficiency leads to a failure in GH secretion from the pituitary and a decline in circulating GH (MacDonald, 2000), but Zn supplementation at 30 mg/kg diet from organic and inorganic sources has no obvious effect on plasma GH concentration and the growth hormone receptor (GHR) mRNA of mice, compared with 11 mg/kg deficient diet in a ten-day period (Yu et al., 2005). Thus, Zn may not directly regulate GH.

Thyroid hormone receptors are members of a large family of receptor proteins. The DNA-binding regions of these receptor proteins all contain nine identically positioned cysteine residues. It appears likely that Zn is required for the biological functioning of thyroid hormones and related receptors (Freake et al., 2001). However, compared with pair-fed rats, the Zn-deficient rats were not significantly different in the concentration of binding protein (receptor) (Freake et al., 2001).

Growth hormone stimulates the synthesis and release of insulin-like growth factor-I (IGF-I), which is essential for longitudinal bone growth as well as amino acid and glucose uptake. Zinc deficiency results in a reduced concentration of IGF-I (Freake et al., 2001; Roughead and Lukaski, 2003). However, dietary Cu is the main determinant of serum IGF-I concentration (Roughead and Lukaski, 2003) and low plasma IGF-I in zinc-deficiency likely results from low food intake rather than from zinc deficiency per se (Browing et al., 1998). Therefore, for the most part, Zn and GH are considered to act independently in regulation of growth and development (Freake et al., 2001).

2.3.4 Immune system

Zinc is also believed to play a role in chick immune function and disease resistance (Underwood, 1977; Scott et al., 1982; Kidd et al., 1996). However, others have shown no influence of dietary Zn concentration on immunological responses in chicks (Stahl

et al. 1989; Pimentel *et al.*, 1991a; Mohanna and Nys, 1999). The immune response of chicks to Zn deficiency is not even clear and immune suppression as a response to varying zinc intake is not as sensitive as decreased growth responses (Pimentel *et al.*, 1991a). It seems the immune response of chicks to Zn deficiency is also due to depressed feed intake.

2.3.5 Intestinal microflora and morphology

A low dietary Zn level is associated with morphological and functional changes in the rat jejunum, which included shortening and narrowing of jejunal villi (Southon *et al.*, 1984). However, even severe restriction of dietary Zn intake has no significant effect on either the size of the intestinal microbial population or on the major colony types isolated. Neither was there any evidence that the increased growth response of the mucosa to subsequent Zn supplementation was linked to an increase in bacterial growth (Southon *et al.*, 1985). This was probably a result of increased cell proliferation (Southon *et al.*, 1984).

2.4 Metabolism of trace minerals

2.4.1 Excretion of trace minerals

Urine is a major route of excretion for some minerals such as Mg, I, and K, but is a minor route for others such as Mn, Fe, Zn and Cu (Ammerman, 1995a). It is known that the gastrointestinal tract (GIT) is a major route of excretion for Cu, Fe, Mn and Zn and apparent absorption is always considered to be limiting.

There is little information about how much of these trace minerals is excreted in faeces of broilers and only one report calculated the whole body concentrations of Cu, Fe, Mn and Zn for broilers to obtain retention values of 6%, 10%, 0.2%, and 6%, respectively, in diets containing less than 20, 190, 85 and 180 mg/kg of these minerals, respectively (Mohanna and Nys, 1998). In another report, it was also shown that the amount of zinc excreted from faeces increased linearly with dietary Zn supplementation (Mohanna and Nys, 1999).

Considering the amount of trace minerals in the excreta, including endogenous minerals that strongly affect actual excretion, perhaps calculating the whole body

concentrations of Cu, Fe, Mn and Zn would be a more accurate way to determine the retention of these trace minerals. Furthermore, it is not known if these retention values would vary with growth rates and dietary levels. There are conflicting reports about the effects of dietary mineral levels on mineral excretion. According to the general model of mineral depletion (Underwood and Suttle, 1999c), during depletion, the storage pools of the minerals are first reduced. However, this model seems to be unsuitable for trace minerals. The model of trace mineral depletion is more likely consistent with the Zn model for laboratory animals (King, 1990). If the dietary deficiency of trace minerals were mild, the bird would reduce the rate of growth and excretion to maintain the normal tissue concentrations. If the deficiency were more severe, the bird would firstly mobilize trace minerals from the exchangeable pool such as the bone. However, so far it is unclear if bone Zn could serve as a store and improve the growth of the birds on a Zn-deficient diet (Giugliano and Millward, 1984). It is necessary to understand the effect of Zn deficiency on bone and body growth of broiler chickens. With such a low retention value, it is possible to consider reducing supplemental trace mineral levels.

2.4.2 Absorption of trace minerals

As mentioned before, the apparent absorption of Cu, Fe, Mn and Zn is poor. Apart from indigestible trace minerals, excreta also contains endogenous excretion from cell abrasion and mineral excess which can not be absorbed. So a suitable supplemental level of trace minerals is crucial to the assessment of trace mineral absorption. An excess of trace minerals always leads to underestimation of the potential trace mineral bioavailability (Underwood and Suttle, 1999c).

Absorption of Cu occurs primarily in the duodenum, although the rest of the small intestine (and perhaps even the colon) is capable of uptake. In studies with rats, actual absorption rates of 30-50% (per day) were reported for intakes within the normal range (Linde, 1996). Uptake efficiency plummets to <10% when intake is excessive.

Practically all of the Fe in the animal's body is organic in nature and there are two kinds of organic Fe, hemal and non-hemal (Henry and Miller, 1995). Hemal and non-hemal Fe are absorbed by different mechanisms. Non-hemal Fe is mainly found in

plants and its absorption is strongly affected by its solubility in the upper part of the small intestine (Yip and Dallman, 1996). Dietary inhibitors of non-hemal Fe absorption include Ca, P, bran and phytic acid. However, the addition of relatively small amounts of vitamin C to the diet increases non-hemal Fe absorption. Hemal Fe comes primarily from haemoglobin and myoglobin in meat and poultry and its absorption is less affected by other dietary constituents. It is unclear if organic Fe is non-hemal or hemal.

Manganese absorption is thought to occur throughout the length of the small intestine and the level of Mn in the diet does not have a pronounced effect on the absorption of minerals (Keen and Zidenberg-Cherr, 1996).

The process of Zn absorption can be physiologically divided into two separate events: uptake of Zn from the lumen into the cell, and Zn transport from the cell into the circulatory system (Swinkels et al., 1994). Generally speaking, the mechanisms of Zn absorption have not been well defined. Although a small portion of Zn uptake and transport may occur through simple diffusion, Zn, a highly charged, hydrophilic ion, is supposed not to cross biological membranes by simple diffusion and its cellular uptake and release are thought be controlled by special mechanisms (McMahon and Cousins, 1998). However, uptake of Zn in the basolateral membrane is not affected by dietary Zn intake, suggesting that Zn absorption is not regulated at the basolateral membrane (Swinkels et al., 1994). Zinc is always associated with another cellular ligand and is not transferred as the free Zn ion, but in the form of a complex with any one or several agents, such as metallothionein (MT), Tris and citrates, etc., that together constitute a distribution system (Jacob et al., 1998).

There is uncertainty about the site of Zn absorption. It is mostly believed that Zn is absorbed by the small intestine, primarily the duodenum and jejunum but also the ileum. Based on a rat model, it was suggested that Zn absorption is much higher in the ileum (approximately 60%) than in the duodenum (19%) and jejunum (20%) (Lonnerdal, 1989). However, absorption of zinc anterior to the small intestine (gizzard) was observed in chickens (Swinkels et al., 1994). Low Zn intake increases the efficiency of absorption. In other words, Zn is absorbed according to need.

Copper, Fe, Mn and Zn are susceptible to hydroxy-polymerization and are acid-soluble (Powell *et al.*, 1999). In the peri-neutral intestinal environment, they readily form insoluble hydroxide precipitates. So most likely the absorption of these trace minerals is regulated in the gizzard by gastric acid output. A marked reduction in gastric acid output may lead to reduced luminal concentration of solubilized and potentially available metal (Powell *et al.*, 1999).

2.4.3 Homeostasis of trace minerals in broiler chickens

The ability to maintain a constant internal state with varying external conditions is essential for survival and is called homeostasis. Changes in trace mineral absorption and excretion in the GIT are primary mechanisms for maintaining trace mineral homeostasis (King *et al.*, 2000). Most dietary Cu in plants is present as complexes with lectins or acidic glycoproteins that are available for extraction by gastric acid. In general, Cu can not be considered a metal that is stored. It usually enters the body from the intestine with ease and is also readily excreted. Copper homeostasis is maintained mainly through excretion. However, it is also believed that adjustment to fluctuations in Cu supply is achieved predominantly by hepatic storage and biliary Cu secretion (Underwood and Suttle, 1999b).

Most of the Fe in the excreta represents unabsorbed dietary Fe (Scott *et al.*, 1982). Iron can be stored in liver and bone marrow. Stored Fe serves as a reservoir to supply cellular Fe needs. Ferritin, one of the storage forms of iron, contains up to 23% of Fe. There do not appear to be any appreciable stores of Mn in the body and Mn homeostasis is maintained by excretion (Keen *et al.*, 2003).

The capacity to store Zn is poorly developed, but significant amounts of Zn may be redistributed from large pools in the bone (Underwood and Suttle, 1999f). A marked reduction in dietary Zn is invariably quickly followed by a reduction in food intake and growth failure (King *et al.*, 2000). The exact mechanism of the low feed intake in Zn deficiency is not known but the reduction in growth associated with Zn deficiency is due mainly to the reduction in intake. It has been demonstrated that when a reduced amount of Zn-adequate diet, equivalent to the amount consumed by similar Zn-

deficient rats was provided, the pair-fed control rats would depress growth in an essentially similar fashion to that of deficient rats (Shay and Mangian, 2000). When the rat is force-fed a Zn-deficient diet by gavage, it will become very sick within a few days. When a Zn-deficient rat was given a choice between a high protein and low protein diets, it would choose the low protein diet (Roth, 2003). However, when Zn-deficient rats were given a choice between carbohydrate, fat and protein, 100% of the reduction in food intake would be due to carbohydrate intake (Shay and Mangian, 2000). Zinc transporters are essential components of systems that influence Zn passage in times of dietary depletion or excess (Liuzzi et al., 2004). Metallothionein is also thought to serve as a mucosal block to Zn absorption and regulates Zn metabolism but it is not a storage protein (Starcher et al., 1980).

It is true that MT can bind Zn, Cu and other heavy metals with high affinity, and the concentration of tissue MT in chickens was positively related to Zn concentration. However, long-term supplementation failed to continue to promote synthesis of MT despite a high level exposure to dietary Zn (Reeves, 1995; Sandoval et al., 1998; Cao et al., 2002). This suggests that MT may act only as an initial defence to prevent excessive Zn absorption and a more efficient mechanism may be adopted when high-Zn intake is prolonged. The mechanisms which enable the animal to maintain Zn homeostasis are not exactly understood and this makes it difficult to assess the Zn status (Swinkels et al., 1994).

2.5 Bioavailability of trace minerals

The bioavailability of trace minerals for broilers is defined as the degree to which the ingested trace minerals in a particular source is absorbed in a form that can be metabolised by broiler birds (Forbes and Erdman, 1983; Sauberlich, 1987; Ammerman, 1995a,b). Currently trace minerals for broilers mainly come from three sources: drinking water, feed ingredients and supplemental inorganic salts or organic trace minerals.

2.5.1 Sources of trace minerals for poultry

Drinking water is not normally a major source of minerals for broilers. Some "hard" water could supply significant amounts of Ca, Mg and S. However, individual daily water consumption is highly variable and it is impossible to calculate mineral intakes from drinking water (Underwood, 1999a). Usually nutritionists ignore the trace mineral contents and their bioavailability in feed ingredients because the requirements of these trace minerals for broilers have always been based on purified diets. Mineral contents, especially trace mineral contents in broiler ingredients, are variable and there is little information about their bioavailability in feed ingredients. Based on limited data, it seems that the bioavailability of these minerals also varies significantly between feed ingredients (Table 2.2). These data suggested that the supplemental trace minerals or the requirements by broilers should be determined on the basis of the diets used for broilers. Trace mineral supplements include inorganic salts such as sulphates, oxides and carbonates, and organically complexed metal ions such as chelates and proteinates, which are considered to be better absorbed and utilized.

Table 2.2 Trace mineral bioavailability in different feed ingredients (%)

Ingredient	Cu	Mn	Zn
Palm kernel meal	44.7	45.7	13.9
Soybean meal	51.0	76.1	66.5
Cottonseed meal	42.3	76.3	38.0
Rapeseed meal	62.2	56.7	57.6
Barley	77.5	54.9	49.1
Wheat	78.5	48.4	57.1
Corn	87.2	60.0	47.5

After AW-Yong *et al.* (1983).

2.5.2 Relative bioavailability of trace minerals to broiler chickens

It is known that the absolute absorption of trace minerals is small and changes in trace mineral absorption and excretion in the GIT are primary mechanisms for maintaining trace mineral homeostasis (King *et al.*, 2000).The apparent absorption of Cu, Fe, Mn and Zn is thought to be not suitable to assess the bioavailability of trace minerals (Ammerman, 1995a), especially when dietary trace minerals have already met or

exceeded requirements of chickens. In addition, bird growth rate is affected by many factors and true bioavailability of trace minerals is difficult to ascertain. So the actual excretion of trace minerals is always ignored and relative bioavailability of trace minerals is commonly used to assess trace mineral status.

The relative bioavailability values are usually calculated by relating poultry response to that of a standard source of the element (usually an inorganic mineral) with an assigned value of "100" (Ammerman et al., 1998). However, this standard source itself was built up depending on the type of biomarkers and the methods used for assessing different trace minerals.

The methods that are used to determine bioavailability of minerals in animal feeds can be divided into two basic classes: balance methods and repletion methods.

- Balance methods include digestibility and retention trials with the endogenous losses estimated by various isotopic labelling techniques. Considering limited success and lack of a radio-isotope with a convenient half-life, the balance method is not satisfactory to determine the bioavailability of trace minerals.

- In repletion techniques, the minerals under investigation are added to a purified or semipurified diet deficient in these minerals and their growth response or the concentration in a suitable tissue is measured. This technique will provide a relative measure of availability and if the treatments include a standard form of the element (usually a soluble inorganic salt) the bioavailability of different sources of the element can be compared to that of a standard. Because semipurified diets are used as the basal diets, the total dietary concentration of the mineral to be tested in the basal diets is less than the requirement (Ammerman, 1995b).

2.6 Assessment of trace mineral relative bioavailability

2.6.1 Biomarkers of relative bioavailability of trace minerals

Growth response

Growth response in the broiler has been used as the primary criterion for determining bioavailability of several trace minerals because the broiler chicken is an ideal assay animal with limited nutrient store, high nutrient demand and rapid growth rate (Ammerman, 1995b). However, one of the difficulties often encountered in trace mineral study is that maximum growth is not obtained because purified diets are poorly consumed (Dewar, 1986). In contrast, when conventional or practical diets were used as the control diets, the amount of trace minerals in the control diet was usually higher than the practical requirement and it is hard to determine the exact requirements of trace minerals for broilers or separate the effect of the supplemental minerals from that of the endogenous minerals in the ingredients (Wedekind et al., 1992). So the key issue for determination of trace mineral requirements or relative bioavailability by broiler chicken growth is the development of semi-purified control diet, in which the trace minerals are deficient but other nutrients will not affect the consumption of diets for birds.

Accumulation of trace minerals in tissues

Accumulation of the mineral element in various target organs has been used for many years as a response criterion (Ammerman, 1995b). Usually tissue weight follows body weight and when expressed relative to body weight, no treatment effects will be observed. However, relative heart and spleen weight are greater in zinc-deficient rats (Shi et al., 1998; Freake et al., 2001). In the past 15 years, the bioavailability of several trace minerals, especially Mn and Cu, has been estimated by tissue uptake of the element following high level, short-term supplementation (Ammerman et al., 1998). The advantages of this method are that it allows formulation of diets with natural ingredients and that the experimental level need not be less than the requirement of the tested mineral. However, the plethoric dosing may affect the excretion of the mineral and could make it more difficult to assess its actual bioavailability. Furthermore, the performance was decreased when the chicks were fed a high concentration of zinc sulphate (Gibson et al., 1986; Jackson et al., 1986; Williams et al., 1989).

It is believed that 85% of the whole body Zn is in muscle and bone (Stefanidou *et al.*, 2006). Zinc concentration in tibia, liver and plasma is usually used as a parameter to assess Zn status. However, Zn deficiency leads to growth retardation, but a relatively normal concentration of Zn in tissues is maintained (Golden, 1988b). Liver Cu accumulation in response to dietary Cu concentration varies with species. Liver Cu concentration began increasing at 100 mg added Cu per kg diet in chicks (Ammerman, 1995b). When dietary Cu level changed from 1.1 to 16 mg/kg diet, Cu concentration in the liver and blood was not sensitive to diet Cu levels (Vahl and Van'Tklooster, 1986; Underwood and Suttle, 1999b). So this is a concentration effect possibly due to excess Cu (100 ppm or greater).

Liver Fe accumulation in response to dietary Fe begins to increase at 280 mg per kg diet. Below 100 mg/kg level, tissue and blood Fe concentrations remain stable (Vahl and klooster, 1987; Aoyagi and Baker, 1995; Underwood and Suttle, 1999d). Manganese concentration in plasma and liver remains at 0.03-0.05 mg/L and 6-12 ug/g, respectively, when dietary Mn level is between 20-50 mg/kg diet. However, Mn concentration in bone seems to be sensitive to diet Mn supplemental levels (Underwood and Suttle, 1999e; Li *et al.*, 2004). When dietary Zn levels are between 8 to 70 mg/kg diet, Zn concentration in plasma only changes from 1.69 to 2.1 mg/L and in liver remains at 53.6 to 55.6 ug/g dry organ (Savage *et al.*, 1964; Dewar and Downie, 1983; Vahl and Van'Tklooster, 1986; Wedekind *et al.*, 1992; Underwood and Suttle, 1999f).

Copper, Fe, Mn and Zn are required for the growth, development and maintenance of healthy bone. Bone ash and bone breaking strength are therefore widely used to measure bone quality of broilers for trace mineral bioavailability. However, some reports did not consider increased trace mineral deposition in bone to be an index for improvement in trace mineral bioavailability (Hunt and Johnson, 1992). Increased or reduced requirements of these trace minerals are still thought to be the only way to assess the bioavailability of trace minerals.

Functional assays

Functional assays for bioavailability in which the mineral element is necessary for an essential compound and stimulates specific enzymes have been used. As previously mentioned, Zn is a component of over 300 enzymes in different species of all phyla (Vallee, 1993). The most widely accepted molecular role for Zn is related to its function as a catalyst in metalloenzyme systems (DiSilvestro and Cousin, 1983). However, human dietary Zn depletion studies have not identified a reliable Zn-dependent enzyme marker of Zn deficiency (Wood, 2000). Furthermore, correlations between the loss of enzyme activities and the characteristics of Zn deficiency have proved unsuccessful and changes in Zn metalloenzymes are not considered the first major causative factor in Zn deficiency that leads to growth depression (MacDonald, 2000).

Recently, Zn and GH have been considered to be acting independently in regulation of growth and development (Freake *et al.*, 2001). Apart from this catalytic role in enzyme systems, Zn is also a component of more than 1000 transcription proteins, suggesting that alterations in mineral status will definitely be translated into changes in gene expression (Dieck *et al.*, 2003; Ho *et al.*, 2003). Zinc is primarily an intracellular ion with intracellular Zn contributing well over 95% of the total body Zn. It is, therefore, important to assess the bioavailability of Zn or other trace mineral elements at a molecular level and investigate the effect of deficiency of trace minerals on functional genes.

In the National Center for Biological Information (NCBI) Gene Bank, there are several peptides, which are suggested to be involved in the regulation of feed intake. Neuropeptide (NPY) has received a lot of attention with regard to feed intake regulation in Zn deficiency but was finally deemed not to be associated with the impaired feeding behaviour in Zn-deficient rats (Reeves, 2003). Cholecystokinin (CCK) is secreted into the blood from endocrine cells in the small intestine upon stimulation by partially digested food and then regulates stomach emptying and feed intake (Reidelberger, 1994), indicating CCK may be a substance involved in feed intake regulation related with Zn deficiency (Reeves, 2003).

On the basis of the foregoing discussion, deficiency of trace minerals, especially Zn, in the diet for broiler chickens can affect growth, enzyme activity and gene expression. A supplement of these trace minerals would prevent retardation of chicken growth, and improve the bone development and tissue accumulation. However, apart from growth rate, there are no clear or specific biomarkers to investigate the deficiency of trace minerals, especially Zn. Current tissue accumulation and functional assays of biomarkers do not seem sufficient to assess trace mineral status.

Upon consideration of Zn homeostasis discussed in Section 2.4.3, when Zn is inadequate to maintain growth or cellular metabolism, reduced food intake may be a protective mechanism to allow survival (MacDonald, 2000). It was demonstrated that animals tended to adapt to low dietary Zn and maintain a relatively normal plasma and tissue Zn concentration (Sullivan *et al.*, 1998). Considering that growth response is the most sensitive biomarker currently and the reduction in growth associated with Zn deficiency is due mainly to reduction in feed intake, the determination of optimal organically complexed Zn supplementation in broiler chicken diets and elucidation of decreased feed intake by dietary Zn deficiency would be crucial to the application of organically complexed trace minerals in broiler chicken production. Otherwise, decreased feed intake also leads to the deficiency of other trace minerals and it is difficult to separate effects from different trace minerals.

2.6.2 Requirements for trace minerals by broiler chickens

The requirement for Cu by chicks was reported by McNaughton and Day (1979) using purified basal diets and a similar method to Davis *et al.* (1968). The chicks gained maximum weight when the supplementary Cu level was 9 mg/kg diet.

Iron requirement by chicks was determined by Davis *et al.* (1968), using body weight, haemoglobin concentration and packed cell volume in the blood as biomarkers, to be 79, 77.5 and 77 mg/kg diet, respectively. However, Vahl and Klooster *et al.* (1987) summarized their previous studies and indicated that the broilers fed practical type diets with more than 80 mg/kg might still be receiving insufficient Fe and they suggested 100 mg Fe/kg diet including its natural content in the ingredients. In their experiments, Davis *et al.* (1968) used isolated soybean meal as the basal diets containing 19 mg Fe/kg diet and the point of interception for body weight response

was 79 mg Fe/kg in the diet. Vahl and Klooster (1987) fed broilers with practical basal diets containing 80 mg iron/kg and added different amounts of inorganic salts up to 1620 mg iron/kg diet and found weight gain response rose to a plateau between 20 and 60 mg added Fe /kg diet. However, neither of these experiments presented data on Fe excretion. It is noteworthy that when the requirements of Fe and Cu were determined, no significant feed efficiency effect was observed.

The Mn requirement of broilers was determined mainly based on the work by Gallup and Norris (1939). In their study a semi-purified basal diet containing 10 mg Mn/kg was used and the supplemented manganese carbonate increased from 20 to 1000 mg/kg diet. At 50 mg/kg, the incidence of perosis was reduced significantly and a growth response was observed, but feed efficiency data were not reported. However, it is reported that based on 37.5 mg Mn/kg diet, there is no further growth response to supplemental Mn levels (Gardiner, 1972).

There is more information about the Zn requirement of broilers, but basically it was determined using semi-purified diets and is approximately 35 to 40 mg/kg diet (NRC, 1994). The growth of chicks fed a semi-purified diet containing soybean protein was significantly increased by addition of zinc chloride to the basal diet (Morrison and Sarett, 1958). Batal et al (2001) also used a semi-purified diet supplemented with 0, 5.81, 10.81 and 15.10 mg Zn/kg from zinc sulphate and found that weight gain of chicks increased linearly over the first three levels of Zn that were fed. Recently it was reported that 80 mg supplemental Zn/kg diet or 110 mg dietary Zn/kg diet supported the optimal body weight gain but there was no significant difference between a basal diet (contain 30 mg Zn/kg diet) and supplemental diets over a 21d period (Burrell et al., 2004).

Currently commercial trace mineral levels are much higher than the requirements recommended by NRC (1994). Figure 2.1 gives some data from the NRC requirements and commercial (Cobb) recommendation (Teeter and Wiernusz, 2003).
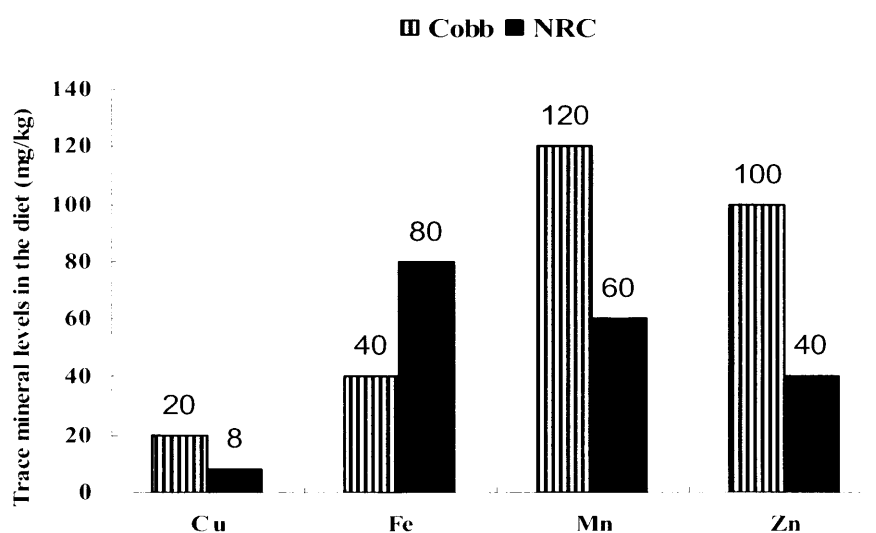
□ Cobb ■ NRC

Figure 2.1 Trace mineral requirements from NRC and commercial recommendations

It is clear that levels recommended by NRC (1994) were based on experiments using semi-purified diets and supplemental trace mineral levels were determined by maximal weight gain. So values were not very high and no feed efficiency or excretion data were reported. In current commercial practice, much higher levels than those recommended by NRC are used. Apart from a consideration for safety margin, nutritionists tend to think that higher levels of trace minerals would better meet the requirements for modern, rapid-growing broiler chickens and possibly promote growth as well. However, it is unlikely that broiler chickens could benefit from these higher levels of trace minerals because there was no difference in bird performance when the bird was supplemented with 10-200 mg Cu/kg diet, 37.9-117.9 mg Mn/kg diet and 40-820 mg Zn/kg diet, respectively (Gardiner, 1972; Min *et al.*, 1994; Aoyagi and Baker, 1995). Thus, these four trace minerals may not act as growth promoters and using higher levels than NRC recommendations may not lead to additional performance. In the higher challenge conditions such as temperatures, diets that exist in commercial situations, extra trace minerals might be beneficial.

2.7 Organic trace mineral supplements for broiler chickens

From an economic and environmental point of view, it is necessary to reduce trace mineral levels in diets close to requirements to minimize waste (Thompson and Fowler, 1990). Organically complexed trace minerals, believed to be better absorbed and utilized than their inorganic salts or oxides, provide a pathway to benefit the environment without compromising bird performance.

2.7.1 Definition of organically complexed trace minerals

Ammerman *et al.* (1998) summarized the following descriptions of organic mineral complexes given by the Association of American Feed Control Officials:

a. A metal amino acid chelate is the product resulting from the reaction of a soluble metal salt with amino acids with a molar ratio of one mole metal to one to three moles of amino acids to form coordinate bonds.

b. A metal amino acid complex is the product resulting from combining a soluble metal salt with an amino acid.

c. A metal proteinate is the product resulting from the chelation of a soluble salt with amino acids and/or partially hydrolyzed protein.

d. A metal polysaccharide complex is the product resulting from complexing a soluble salt with a polysaccharide solution declared as an ingredient as the specific metal complex.

Unlike inorganic salts, where the metal ion is bound by electrovalency, organic trace minerals are metal complexes that are coordination compounds. In these metal complexes the donor atoms donate electrons to an acceptor metal atom, and once this donor-acceptor complex is formed, it is very difficult to distinguish this coordinate bond from a covalent bond (Kratzer and Vohra, 1986). In contrast, inorganic trace mineral salts easily release free ions which are very reactive and can form complexes with other dietary molecules that are difficult to absorb in the animal's intestinal tract (Close, 1999). Further, these free ions can interact with each other and affect enzymatic activities. However, there is evidence that metal complexing may help solve these problems. Recently the metal chelate has been shown to traverse the mucosal cell membrane, the mucosal cell and basement membrane, still intact, into the circulation (Power, 2003).

Both peptides and free amino acids are absorbed from the GIT and serve to fulfil the needs of the animal for tissue growth and maintenance, but peptides are a more important form of amino acid absorption than free amino acids (Rerat and Simoes-Nunes, 1988; Webb *et al.*, 1992). Peptide and free amino acid transport is now understood to be distinctly separate physiological processes involving different transport proteins (Webb *et al.*, 1992; Webb *et al.* , 1993). Although there are four types of organic metal complexes currently used in practical and experimental animal nutrition, evidence suggests that the metal proteinate shows more efficiency than the other types.

2.7.2 History of organically complexed trace minerals for broilers

As early as 1959, it was found that the availability of Zn in soybean protein was greatly improved by addition to the diet of an efficient chelating agent. Then it was demonstrated that chickens on the diet with added liver extract containing only 1.5 mg Zn/kg diet, in the presence of 10 mg Zn/kg diet in the basal diets, reached similar weight gain in chickens that received 60 mg Zn/kg diet (Scott and Zeigler, 1963). However, in the said experiment the body weight of birds only reached 500 g in 4 weeks of age with Zn supplementation. It is not clear if 11.5 mg Zn /kg diet already met the requirement for growth or if the liver extract improved Zn utilization.

In 1972, it was reported that Zn from corn was 40% more effective than the inorganic supplement at increasing tissue levels of the minerals (Patton, 1997). Since then, over 100 references have been made to the bioavailability of organic trace minerals and have implied that the advantages of organic trace minerals over inorganic supplements will not be necessarily evident in all circumstances.

Since organic complexing of trace minerals may protect trace minerals from phytate and other factors in the chicken diet, and modern fast-growing broiler chickens may need more trace minerals than those before, much research from the 1990s onwards has focused on the effect of organic trace minerals on broiler and other animal performance. The outcome of this research indicates that it is possible to use suitable organic trace mineral supplements only for broiler chickens. Such a practice saves resources and reduces environmental pollution.

2.7.3 Attributes of organic mineral supplements

There have been few experiments that used semi-purified diets to assess bioavailability of organic trace minerals for broilers in general. Most studies employed basal diets using commercially available ingredients that would have contained enough trace minerals to meet the requirements of broilers (Henry et al., 1989; Hess et al., 2001; Lee et al., 2001; Paik, 2001; Chowdhury et al., 2004; Revy et al., 2004). On the basis of chicken performance, so far there is no direct evidence to demonstrate that the organic trace minerals would be more effective than inorganic salts. However, based on trace mineral accumulation in animal tissues, organic trace minerals did show their advantage over or at least are as efficacious as inorganic salts.

Organic Cu

A soybean meal basal diet containing 0, 500 and 750 mg Cu/kg diet from copper sulphate was supplemented with 0 and 0.06% copper methionine for turkey poults. Added Copper-methionine (Cu-Met) improved body weight, feed consumption and feed efficiency significantly (Harms and Buresh, 1987). Aoyagi and Baker (1993) used purified diets to demonstrate that relative bioavailability of copper-lysine on the basis of bile Cu concentration was estimated as 120% compared to copper sulphate, but this was not statistically significant. Guo et al. (2001) added 150 and 300 mg/kg of different sources of Cu to a basal diet containing 16 mg/kg Cu and concluded that the organic Cu has a greater bioavailability. It is interesting to note that the bioavailability of copper proteinate relative to copper sulphate has been calculated as either 147% or 112%, depending on whether liver Cu or plasma Cu is used as the response criterion (Kincaid et al., 1986; Baker and Ammerman, 1995). Such findings are in agreement with recommendations that liver Cu levels, as opposed to plasma levels, are a better indicator of Cu status and relative bioavailability between sources (Lee et al., 1988; Xin et al., 1991).

From the foregoing review, it can be deduced that the bioavailability of organic Cu was mainly assessed by Cu accumulation in liver and bile tissue based on high level of supplemental organic Cu. However, in practice, such a high supplemental copper level is rare in broiler chicken diets. It is unclear if organic Cu supplemented at a lower level is still superior to its inorganic salt.

Organic Fe and Mn

Few studies, especially using purified diets, have been conducted with organic Fe for broilers, and there is no published literature to show that organic Fe is more available than inorganic Fe. Fly et al. (1989) suggested that for chicks, Mn in the form of Mn-methionine was more available than that from the oxide. Henry et al. (1989) demonstrated that, based on slope ratios from multiple linear regressions of bone and kidney levels, Mn concentrations differed depending on the sources of added Mn. The relative bioavailability values were 96% and 86% from Mn oxide and 108 and 132% from Mn-methionine on the bone and kidney models, respectively, compared with 100% from manganese sulphate. In addition, Virden et al. (2004) used broiler breeders fed supplemental organic Zn and Mn to demonstrate increased concentrations of Zn and Mn in the left ventricle, septum and total ventricular weights for the progeny chicks.

Organic Zn

Pimentei et al. (1991b) compared zinc-methionine with zinc oxide for chicks and concluded that the bioavailability of Zn from the two sources appeared to be similar. However, they also indicated that chicks fed zinc-methionine had a greater concentration of Zn in the pancreatic tissue than chicks fed zinc oxide when 28 and 58 mg Zn/kg diet were fed. The concentration of Zn in the liver of chicks fed zinc-methionine also tended to increase.

Using a purified diet containing 13 mg Zn/kg diet and supplementing inorganic and organic Zn at 0, 7.5 and 15 mg/kg diet, respectively, Wedekind et al. (1992) reported that the bioavailability of zinc methionine (Zn-Met) for chickens was 124% and 177% relative to that of zinc sulphate, on the basis of weight gain and total tibia concentration, respectively. In their experiment, birds fed Zn-Met consumed more feed than those fed inorganic salts and this resulted in increased consumption of both supplemental Zn and basal Zn. Because Zn bioavailability in the basal diet was not assessed at that stage, it can not be determined whether the weight gain and bone zinc response were attributable to supplemental Zn (Wedekind et al., 1992). This report did not provide an estimate of the organic Zn requirement and obviously 28 mg Zn/kg diet could not support optimal broiler growth.

On the basis of 55 mg Zn/kg in the mineral premix and 20 mg Zn/kg in the control diets, Hess *et al.* (2001) compared Zn influence from lysine complex and methionine complex with the control diets. Although the feed conversion efficiency was reduced in female broilers, body weight of male broilers at 21 d was improved.

When Zn proteinate was added at 30, 60 and 90 mg/kg to a basal diet for chicks, the bone Zn concentration was greater than that for inorganic Zn (Cao *et al.*, 2002). Dozier *et al.* (2003) evaluated diets containing 4, 8, 12 mg Cu /kg and 40, 80, 120 mg Zn/kg from different sources. Birds that were provided feeds supplemented with increasing concentrations of Zn from organic sources had a higher toe ash Zn content although there was no difference in body weight, feed conversion ratio or total mortality. Birds provided with diets supplemented with 40 mg Zn/kg had a lower amount of Zn excretion. However, this experiment did not test lower amounts of organic Zn or include control group, and the excretion was evaluated by total collection over only one 48-h period.

2.7.4 Limitation to the use of organic trace mineral supplements

On the basis of the preceding review, organic trace minerals appear to provide a pathway to minimize the trace mineral levels in the excreta and meet the broiler chicken requirements for optimal growth and health. However, due to the lack of specific biomarkers for assessment of trace mineral bioavailability, the advantages of using organic trace minerals have not been established definitively.

For example, Pott *et al.* (1994) used a basal diet supplemented with 150, 300 or 450 mg Cu/kg from organic and sulphate sources to feed broiler chickens for 18 days. Based on liver Cu levels there was no difference in bioavailability between inorganic and organic sources of Cu. On the other hand, using the slope ratio of liver iron concentrations, high supplemental levels of 400 to 800 mg/kg diet from iron-methionine complex, based on a practical basal diet, a bioavailability of 88% was obtained compared to ferrous sulphate (Cao *et al.*, 1996).

Baker and Halpin (1987) fed male chicks a casein-dextrose basal diet containing 1.4 mg Mn/kg to which 1000 mg Mn/kg was added from manganese sulphate or

manganese proteinate. Manganese bioavailability was not significantly different from the two sources. However, as the level of 1000 mg Mn/kg diet in this experiment is far in excess of the chicks' requirement for Mn, it is unclear whether a lower amount of organic Mn added to a purified diet could lead to a similar result. In another experiment practical diets containing 118 mg Mn/kg and supplemented with 0, 500, 1000 and 1500 mg Mn/kg from amino acid chelates and inorganic salts were fed to chicks (Miles *et al.*, 2003). Based on the bone Mn concentration regressed on added dietary Mn concentrations, the relative bioavailability was 84% for the Mn chelate. Mohanna and Nys (1999) fed chickens basal diets containing 20 mg Zn/kg and added 10, 25, and 40 mg/kg of different sources of Zn for 21 d. No differences were found between organic and inorganic sources. They indicated that 10 mg supplemental Zn/kg diet already supported the best FCR.

It was reported recently that birds fed basal diets containing 30 mg Zn/kg and supplemented with 20, 40, 80 mg/kg of different sources of Zn for 45 d linearly increased body weight but there was no difference between Zn sources (Burrell *et al.*, 2004). However, in this report the regression equation was not shown and it is hard to compare with other results.

From the foregoing discussion, it is clear that most experimental results regarding organic trace minerals, primarily based on trace mineral accumulation in tissues at much higher levels than practical usage, showed a benefit, or at least no disadvantage, to inorganic trace minerals. The accumulated data suggest that the basal diets used in most experiments may obscure the responses because of the poor intake of nutritionally unbalanced semi-purified diets or because practical diets already exceed the requirements for some or all of the trace minerals being measured. Further, there are questions over the methodology used in some determinations of the efficacy of organic trace minerals, particularly those experiments where very high concentrations of minerals are added to diets already containing adequate levels of the test minerals. It is also clear that these high levels of trace minerals for bioavailability assessment may not be appropriate for supplementation in practical chicken production. Due to unsuccessful functional assays and relatively consistent tissue contents, growth response and total tibia contents appear to be the most reliable biomarkers for the efficacy of trace minerals, especially Zn, where lower levels of trace minerals are

used. Considering that the current requirements of all these trace minerals were determined by growth response but not by tissue accumulation, it is necessary to determine the optimal supplemental levels of organic trace minerals for practical broiler production by the latter assay.

2.7.5 Factors affecting the utilization of organic trace minerals

The trace mineral requirements of broiler chickens are easily met, but often ignored in commercial practice. They are present in chicken tissues in very small, constant amounts, but play important roles in chicken growth. There are several factors that may affect the utilization of trace minerals from organic complexes.

Age of bird

The efficiency of utilization of organic Zn, for example, decreases as the animal's age increases, suggesting that there may be less benefit in using organic Zn sources in foods for adult animals (Smith et al., 1995). This is probably due to increased diet uptake and hence the intake of trace minerals, as animals mature. However, it may also be due to the immature biliary function in young animals, which may tend to reduce endogenous Zn excretion.

Basal diets and phytate

Early investigations of the requirements of most trace minerals by broiler chickens were based on purified diets and there was a tendency to ignore the mineral contents of the basal ingredients because of the uncertainty or inaccuracy of the data from books and the lack of real data on local ingredients. However, it is still noteworthy that dietary ingredients affect the bioavailability of trace minerals whether the chickens are fed purified diets or practical diets. For example, the requirements determined for Fe, Mn and Zn are much lower for chicks fed purified diets than for those fed practical diets. The NRC ascribed this phenomenon to relatively poor bioavailability of some minerals in practical ingredients (NRC, 1994). In practical diets, many plant ingredients such as soybean, contain components, like phytate, that complex with Cu, Zn and other minerals, affecting their bioavailability (Davis and Kratzer, 1962). Conversely a high level of phytate was also considered to enhance Cu availability by reducing the competition from plant Zn (Wappnir, 1989).

However, it is believed that when the diet is provided with organic Zn, the phytate probably does not reduce this competition. The bioavailability of inorganic Mn seems not to be influenced by phytate. Manganese sulphate retained a high absorbability of 10.3% when added to a maize-soybean meal diet, in which only 2.8% of the inherent Mn was absorbed by chicks (Wedekind *et al.*, 1991).

As mentioned before, purified diets are poorly consumed (Dewar, 1986) and can not support the bird to reach its growth potential, leading to compromised growth of the chick due to deficiency of other nutrients (Wedekind *et al.*, 1992). Therefore, increased requirements of trace minerals in practical diets may also result from increased growth rate due to practical diets.

Dietary protein contents

A high protein diet significantly increased the apparent absorption of Zn and deposition in bone (Snedeker and Greger, 1983), but Zn requirement by animals fed a high protein diet did not decline due to this improved Zn bioavailability (Hunt and Johnson, 1992). In contrast, animals fed a low protein diet had a reduced Zn requirement. For example, rats fed diets with extremely low protein levels had a poor feed intake but no visible signs of Zn deficiency. With increasing dietary protein levels, the incidence of signs of Zn deficiency rose in the zinc-depleted rats (Roth, 2003). Therefore, Zn requirement may be determined by animal growth rate but not by dietary protein level.

Dietary Ca, P and Environmental temperature

Calcium retention increased with the addition of Zn to swine diets, but Zn had no influence on P retention, nor was retention of absorbed Zn affected by increased dietary Ca (Morgan *et al.*, 1969). Most studies on the biological availability of trace minerals have been conducted in temperature-controlled environments suitable for bird growth. However, high environmental temperatures have been shown to increase mineral excretion (El Husseiny and Creger, 1981; Smith and Teeter, 1987; Belay *et al.*, 1992). Under heat-stress, organic Mn significantly increased tibia Mn concentration and showed greater bioavailability than that from $MnSO_4$ (Smith *et al.*, 1995).

Mineral toxicity and inorganic sources

There are various parameters to investigate the bird's response to the incorporation of inorganic trace minerals: the minimal levels associated with deficiencies; optimal levels where requirements are met; and maximal levels which would result in toxicity (Table 2.3) (Larbier, 1992).

Table 2.3 Minimal, optimal and maximal levels of trace minerals in the diet for broilers (mg/kg diet)

	Optimum	Minimum	Maximum
Copper	8	4	250
Iron	80	35	1000
Manganese	60	45	600
Zinc	50	40	800

After Larbier (1992)

Obviously there are wide ranges between optimal and toxic levels of Cu, Fe, Mn and Zn in broilers but relatively narrow ranges between minimal and optimal levels. To determine the requirements of more available organic trace minerals, in basal diets, Cu, Fe, Mn and Zn contents are better maintained at 4-8 mg, 35-80 mg, 45-60 mg and 40-50 mg, respectively, in order to produce deficiency syndrome. There is evidence that organic trace minerals are a lot less toxic than inorganic minerals (Scott *et al.*, 1982). This is believed to be due to the fact that these organic minerals are transported via active transport routes whereas inorganic minerals are usually absorbed through a concentration gradient, which is easily converted to insoluble compounds.

2.8 Interactions between trace minerals

Interactions among minerals are a major cause of variation in availability and thus influence the nutritive value of a diet. Underwood and Suttle (1999a) described three types of mineral interactions: the formation of unabsorbable complexes between dissimilar ions in the gut (*e.g.*, metal phytates); competition between similar ions for metabolic pathways; and induction of non-specific metal-binding proteins. While organic trace minerals protect minerals from phytate or other anti-nutritive dietary factors in relatively small amounts, it is still possible for similar trace mineral ions to

compete for transport systems and metabolic pathways. It is worthwhile examining a few notable interactions.

2.8.1 Cu and Fe

Although the recommended quantities of Fe and Cu vary among researchers, the ratio of dietary Fe to Cu shown in the literature is most commonly recommended with a 10:1 ratio (Mcnaughton and Day, 1979). The absorption of Cu is not affected by Fe status, but Fe absorption is significantly lowered by Cu deficiency (Schwartz, 1974).

2.8.2 Zn and Fe

Iron and Zn interact at the level of the intestinal mucosa and Zn absorption is impaired by Fe (Solomons and Jacob, 1981). Excess Zn interferes with Fe incorporation into ferritin (Settlemire, 1967).

2.8.3 Zn and Cu

Excess Cu decreases Zn absorption significantly (O'Dell, 1984). Excess dietary Zn is antagonistic to Cu and leads to anaemia. Copper status is highly sensitive to Zn intake, much more so than the reverse interaction (Baker and Ammerman, 1995). It is interesting that a reduced dietary Zn supply enhances uptake and absorption of Cu and Zn as well (Cousins, 1985). While phytate, which is rich in practical diets, was considered to enhance Cu availability by reducing the competition presented by inorganic Zn. It is unknown if organic Zn could prevent this competition.

2.8.4 Mn and Fe

Excessive dietary Mn decreased haemoglobin content (Baker and Halpin, 1991). So far, no interaction between Zn and Mn has been established.

One of the major reasons put forward for the use of organic minerals is that they are protected from these interactions. Thus, it is necessary to reconsider the requirements by the broiler chickens when broiler diets are supplemented with organic Cu, Fe, Mn and Zn.

2.9 Importance of the current research

There is limited knowledge of the use of organic Cu, Fe, Mn and Zn in practical broiler chicken production. Due to difficulties in direct assessment of the bioavailability of these trace minerals, current research investigating the application of organic Cu, Fe, Mn and Zn in broilers, mostly based on tissue trace mineral accumulation in much higher dietary trace mineral levels, has not provided clear results for the optimal supplemental levels of these organic trace minerals, especially Zn. The absorption and excretion of organic trace minerals are still not clear and further work is necessary to better understand conflicts between environmental benefits and optimal meat chicken production.

2.9.1 Safety margins and environmental pressure

In commercial practice nutritionists use higher levels than those recommended by the NRC and it has been considered safer to overfeed by appreciable margins. This trend leads to increased cost and an elevated level of mineral excretion. High rates of mineral excretion in slurry and manure pollute drainage water or affect mineral metabolism in plants. Organic Cu, Fe, Mn and Zn may offer a solution to this challenge.

2.9.2 The growth rate of the modern broilers

Currently, the body weight of a meat chicken reaches 2 kg in 35 days, down from 64 days in 1979, but Cu, Fe, Mn and Zn requirements by broilers are still thought to be at the same level as those recommended by NRC in the 1990s (NRC, 1994). It is reasonable to consider that the current NRC values would not meet the needs of the modern birds. It has been suggested that the mineral needs for growth in the modern fast-growing pig are about twice the level required by the slower growing pig of some 20-30 years ago. So it is necessary to determine whether older NRC requirements are still appropriate for today's birds.

2.9.3 The optimal requirements of organic Cu, Fe, Mn and Zn

From the foregoing discussion, growth response is the most sensitive method to evaluate trace minerals, especially Zn requirement. Therefore, it is reasonable to find some practical ingredients containing lower levels of trace minerals to incorporate

into a control diet that will allow birds to reach genetic potential of growth when tested trace minerals are supplemented. From the bird growth response, it is possible to determine an optimal amount of organic trace minerals, especially Zn, for broilers.

2.9.4 Absorption of organic trace minerals

Currently there are conflicting results about absorption sites for Cu, Fe, Mn and Zn. Considering that inorganic salts of these trace minerals are susceptible to hydroxy-polymerization and are acid-soluble, they readily form insoluble hydroxide precipitates in the intestinal environment (Powell et al., 1999). In contrast, organic Cu, Fe, Mn and Zn are believed to traverse the mucosal membrane in the intestine and still intact, into the circulation (Power, 2003). So it is necessary to study the absorption sites for organic trace minerals. By collecting digesta from different parts of the intestinal tract, analysis of the mineral contents compared with a suitable marker, may indicate the absorption site of organic trace minerals.

2.9.5 Bone quality and organic trace minerals

Although Mn and Zn concentrations in the tibia are considered to be sensitive to the dietary trace mineral levels and have been used as a biomarker to assess the bioavailability of organic trace minerals, there is no report for the practical usage of organic trace minerals on bone quality and trace mineral concentrations. In addition, there is no hard evidence to show that trace minerals, especially Zn, could be deposited in the bone and improve growth of birds on a zinc-deficient diet. So it is necessary to investigate the effect of trace minerals on tibia quality, looking at breaking strength and the depletion effect on mineral concentration in tibia.

2.9.6 Mineral interaction and molecular biological approaches to the regulation of feed intake by the deficiency of Zn

It has been shown earlier that a large body of literature indicates that deficiency of Zn strongly affects the feed intake of birds, but there is no report that other trace elements are involved. However, fluctuations in feed intake due to zinc deficiency will definitely affect the intakes of other trace mineral. Considering that organically complexed Zn has the potential to be absorbed better, and that it may have a different metabolic pathway to the inorganic salts, it is necessary to define its molecular

mechanisms to guide the application of these products in broiler chicken production. There are several peptides that are suggested to be involved in the regulation of feed intake and CCK may be a substance involved in feed intake regulation in association with Zn deficiency (Reeves, 2003).

2.10 Conclusion

Copper, Fe, Mn and Zn are essential for broiler chicken growth. In commercial practice, the use of very high safety margins for these inorganic trace minerals in broiler feed formulation has resulted in a high level of mineral excretion. Organic trace minerals are generally thought to be better absorbed and utilized than their inorganic salts or oxides, providing a possible means to solve this problem. However, due to difficulties in assessment of the availability of these trace minerals, most previous research on organic trace minerals is based on tissue trace mineral accumulation using very high dietary trace mineral levels. Hence, although organic trace minerals have shown advantages or at least no disadvantage over inorganic salts, there is a gap between the levels used in practice and those used in laboratories to assess requirements or set recommendations. It is necessary to determine suitable supplemental levels of organic trace minerals for broiler chickens.

Based primarily on genetic selection for feed intake, the body weight of today's fast-growing broiler chickens can reach 2 kg within 35 days. However, Cu, Fe, Mn and Zn requirements of broiler chickens, all determined by purified or semi-purified control diets 20 to 30 years ago, probably do not support optimal chicken performance. In addition, the deficiency of Zn strongly depresses bird feed intake, leading to deficiency of other trace minerals and nutrients, it is necessary to determine the optimal organic Zn requirement and any interaction between these trace minerals in order to allow the bird to reach genetic growth potential but reduce trace mineral excretion.

One of the difficulties often encountered in the determination of optimal trace mineral requirement is that maximum growth may not be achieved because purified diets are poorly consumed. So the key issue for determination of trace mineral requirements by broiler chicken growth is that development of semi-purified control diet, in which the

trace minerals are deficient but other nutrients will not affect the feed intake of birds.

Because the homeostasis of Zn, Cu, Fe and Mn is controlled by absorption and excretion, direct assessment of trace mineral bioavailability, especially at practical supplemental levels, is impossible. Evaluation of trace mineral absorption sites and molecular mechanisms of feed intake regulation by Zn will be an attempt to identify any difference in uptake of trace minerals between organic and inorganic sources.

CHAPTER 3. General Materials and Methods

3.1 Experimental methods

3.1.1 Bird husbandry

All experiments reported in this thesis involved one-day-old male commercial broiler chickens (Cobb), which were obtained from a local hatchery (Baiada hatchery, Kootingal, NSW). The birds were raised in brooder units (42 cm × 75 cm × 25 cm dimension) with wire floor or floor pens (120 cm × 120 cm × 90 cm) with wood shavings as litter. They were given experimental starter diets from d 1 to d 14. The brooding temperature was gradually decreased from approximately 34 °C at d 1 to 25-28 °C at d 14. Water and feed were provided *ad libitum*. From d 14 to d 35, the birds were fed finisher diets and the birds were transferred into metabolism cages situated in well-ventilated rooms. During the finishing period, the room or shed was kept at a constant temperature of 23-25° C. The birds were maintained under a 20 h light:4 h dark program.

3.1.2 Experimental diets

All the experimental diets were formulated using mainly sorghum and isolated soy according to the recommendations of the NRC (1994) with the exception of Cu, Fe, Mn and Zn, which were added to the experimental diets separately. The sorghum was hammer-milled. The isolated soy and other granular additives were added directly to other ingredients according to the supplier's recommendation. All minor ingredients, including organic and inorganic trace minerals, were diluted and hand-mixed before combining with the major ingredients for thorough mixing in a horizontal mixer. All diets were cold-pelleted (approximately 60 °C). Vegetable oil was sprayed on the other ingredients during mixing.

The isolated soy was purchased from ADM (Archer Daniels Midland Company Australia Pty Ltd, Queensland, Australia). Organically complexed trace minerals (Bioplex Cu, Fe, Mn, Zn, Alltech Biotechnology Pty Ltd, Dandenong South, Victoria, Australia), feed-grade sulphate trace minerals (CuSO$_4$, FeSO$_4$, MnSO$_4$ and ZnSO$_4$) and vitamin-trace mineral premix, free of Cu, Fe, Mn and Zn (DSM Nutritional

Products Australia Pty Ltd, French's Forest, NSW, Australia) were kindly provided by the manufacturers.

Table 3.1 Composition of commercial broiler vitamin/mineral premix (inclusion rate 1 kg per tonne of feed, supplied by DSM, vitamin and mineral specification provided per kg of diet)

Ingredients		Ingredients	
Vitamin A	10,000 IU	Pyridoxine (Vitamin B_6)	7.00 mg
Cholecalciferol (Vitamin D_3)	2,500 IU	Cyanocobalamin (Vitamin B_{12})	25.00 µg
Vitamin E	50 mg	Biotin (Vitamin H)	250 .00 µg
Thiamine (Vitamin B_1)	2 mg	Se (Selenium)	0.30 mg
Riboflavin (Vitamin B_2)	10 mg	I (Iodine)	1.00 mg
Niacin (Vitamin B_3)	50 mg	Mo (Molybdenum)	0.50 mg
D-calcium pantothenate	7 mg	Co (Cobalt)	0.25 mg

3.1.3 Balance trial and apparent metabolizable energy (AME) bioassay

Balance trials were conducted over a period of eight days. Prior to the actual faeces collection period, birds were fed the experimental diets for 3 d as an adaptation period. During the following 4 d, all faeces-collecting trays were covered with a piece of plastic sheet and water troughs were changed to plastic containers, containing distilled water. Feed intake was measured and all excreta were collected daily. The excreta were dried in a fan-forced oven at 80° C for 48 h and excreta from each replicate were pooled over the collection period for determination of trace mineral concentrations. The moisture content of the excreta was also measured.

Apparent metabolizable energy (AME) was conducted over a period of 7 days during the fourth week of the experiment (21 to 28 d post-hatching). Acid insoluble ash

(AIA) was used as a marker. Clean excreta trays, covered with (pieces of) plastic sheets were placed under each AME cage, excreta were collected daily, dried at 80° C to a constant weight in a forced-drought oven. Collections were pooled within each cage for analysis. Care was taken to avoid contamination with feed, feathers and debris. The moisture content of the excreta was measured. Diet and excreta were ground to pass through 0.5 mm screen using a stainless steel blade grinder. Gross energy of diets and excreta were determined using an IKA bomb calorimeter (IKA®-WERKE, C7000, Staufen, Germany) standardized with benzoic acid. AME of diets was calculated using the equation:

$$AME_{diet}(MJ / kg\ DM) = GE_{diet} - \left[GE_{excreta} \times \left(\frac{AIA_{diet}}{AIA_{excreta}} \right) \right]$$

where, GE_{diet} = Gross energy in diets

$\qquad GE_{excreta}$ = Gross energy in excreta

$\qquad AIA_{diet}$ = Acid-insoluble ash in diets

$\qquad AIA_{excreta}$ = Acid-insoluble ash in excreta

3.2 Analytical techniques

3.2.1 Sample collection and process

After pelleting, about 500 g diet samples from starter and finisher diets were collected for grinding and then kept in plastic containers for analysis.

During the balance trial, the AME trial and the depletion-repletion trials, all excreta were collected into aluminium trays and placed into a forced-drought oven for at least 48 h and then ground in a stainless steel grinder. The excreta were then stored in plastic containers for analysis. Sampled birds were killed on d 21 and d 35. One or two birds from each replicate were randomly selected and euthanized by cervical dislocation.

Before killing, blood samples were obtained from the jugular vein and collected into heparinized tubes individually. The tubes were then immediately centrifuged at 1000×g for 15 minutes (Allegra ^{TM}6R, Beckman, USA) and the supernatant was transferred to 5 mL tubes and frozen at −20 ° C for analysis.

The whole chicken samples, tibia bone and liver samples that were removed from birds, were put into plastic bags and immediately frozen at -20 ° C for analysis.

The body cavity was opened and the contents of the gizzard, duodenum, jejunum (from duodenum to the Meckel's diverticulum) and ileum (from the Meckel's diverticulum to 4 cm above the ileo-caecel junction) were collected and stored in plastic containers and frozen immediately at $-20°$ C for analysis. The liver, heart and spleen were removed and weighed.

To prepare the small intestine and pancreas samples for real-time PCR, the jejunum was emptied and the pancreas were removed and rinsed in cold distilled water. About 100 mg samples were taken from the pancreas and middle-part of the small intestines were collected into 2 mL Eppendorf tubes and snap-frozen in liquid nitrogen, then stored at $-80°$ C for analysis.

3.2.2 Routine analyses

Dry matter

The dry matter (DM) content was determined gravimetrically following drying at $105°C$ in a forced-air convection oven (Qualtex Universal Series 2000, Watson Victor Ltd, Perth, Australia) for 24h (for diets, bone, liver tissue) or drying at $80°$ C for 48h (for excreta) or freeze-dried (Marttin Christ freeze dryer, Germany) at $-50°C$ (for digesta and whole body of chickens).

Gross energy

Gross energy contents of diets and excreta were determined using an IKA bomb calorimeter (IKA^R-WERKE, C7000, Staufen, Germany).

Crude protein

The nitrogen content of diets and excreta was determined according to the Dumas combustion technique following the method described by Sweeney (1989) using a LECO^R FP-2000 automatic nitrogen analyzer (Leco Corp., St Joseph, MI, USA).

Crude ash

Dry tissue, tibia and whole chicken meat were weighed accurately and placed into

crucibles, then ashed at 550 °C for 4 h in a Carbolite CWF 1200 chamber furnace (Carbolite, Sheffield, UK). Furnace temperature was set at 200 °C initially and then rose to target temperature after one hour. The residue was weighed and crude ash content was calculated.

Mineral analysis

Standard solution: Stock solutions of different mineral were prepared (Stock A). From these a second set of stock solutions (Stock B) were made. Different amounts of stock B were pipetted and analysed to derive a standard curve. Based on different digestion systems, nitric acid, hydrochloric acid and perchloric acid were added to analyse for mineral contents in diets, excreta and digesta or tibia bone ash, respectively

Measurement: diluted samples and standard solution were separately put into a pack of fresh tubes for inductively coupled plasma emission spectroscopy (ICP) (Vista MPX, Melbourne, Australia). Copper, Fe, Mn, Zn, Ca, P were measured at 327, 238, 257, 213, 616, 214 nm wavelength, respectively.

3.3 Statistical analysis

Statistical analyses were performed using STATGRAPHICS software (Manugistics, Inc., Rockville, Maryland, USA). The data were analyzed using one-way ANOVA with diet as factor. The significance of difference between means was determined by Duncan's multiple range test. Regression analysis was carried out between the control group and organic supplemental groups using polynomial regression model or multiple regression model with supplemental zinc intake and control dietary zinc intake as the independent factors.

3.4 Animal ethics

The Animal Ethics Committee of the University of New England approved the studies reported in this thesis. Health and animal husbandry practices complied with the "Australian code of practice for the care and use of animals for scientific purposes" issued by the Australian Government National Health and Medical Research Council (AGNHMRC, 2004).

CHAPTER 4. Effect of Organic Cu, Fe, Mn and Zn on Broiler Performance, Mineral Excretion and Accumulation in Tissues

Abstract

Supplementation of trace minerals with a large safety margin in broiler chickens has resulted in a high level of mineral excretion which ends up in the environment. Organic trace mineral supplements may be able to replace the inorganic supplements as the former appear to have a greater bioavailability. Therefore, a 29-day cage study that included diets with supplemental trace minerals from organic and inorganic sources based on a trace mineral deficient control diet was conducted to examine the possible response of broiler chickens to organic mineral supplements. The results showed that the deficiency of Cu, Fe, Mn and Zn strongly depressed (P<0.01) feed intake and therefore body weight gain but did not affect (P>0.05) trace mineral accumulation in tissues. Thus, supplementation with 4 mg Cu and 40 mg each of Fe, Mn and Zn per kg from organic sources may be sufficient for normal broiler growth to 29 d of age. It is possible to use these lower levels of organic trace minerals in broiler diets to avoid high levels of trace mineral excretion.

4.1 Introduction

Trace minerals, such as Cu, Fe, Mn and Zn, are essential for broiler growth and are involved in a large number of digestive, physiological and biosynthetic processes within the body. They function primarily as catalysts in enzyme systems within cells or as parts of enzymes. They are also constituents of hundreds of proteins involved in intermediary metabolism, hormone secretion pathways and immune defence systems (Dieck *et al.*, 2003). Traditionally these trace minerals are supplemented in the form of inorganic salts, such as sulphates, oxides and carbonates, to provide levels of minerals which prevent clinical deficiencies and/or allow the bird to reach its genetic growth potential.

Despite enormous advances in poultry production and technology, research into trace mineral nutrition has lagged behind other areas of nutrition. Currently the body weight of a meat type chicken reaches 2 kg in 35 days, down from 64 days in 1979. However, the trace mineral requirements for broilers have still been thought to be the same level as those recommended by the National Research Council for Animal Nutrition in the early 1990s (NRC, 1994), some of which are based on data as far back as the 1950s. Although most of the increase in body weight via genetic selection has been an indirect response to selection for appetite, increased body growth has resulted in skeletal problems, which may be related to poor mineral nutrition. It is thus reasonable to consider the current NRC recommendation as unsuitable for the needs of the modern bird.

Actually the industry is still using a large safety margin in feed formulation on account of higher dietary mineral needs and cheaper cost of trace mineral sources. So in commercial practice, these supplemental inorganic trace minerals result in a high level of mineral excretion. Obviously this is not only wasteful, but also harmful to the environment. For example, poultry manure applied on a nitrogen basis contains zinc and copper 660 and 560%, respectively, in excess of crop requirements (Dozier *et al.*, 2003). Due to the concern for build-up of heavy metals when applying poultry litter to cropland, environmental protection agencies around the world have pressed for lower levels of mineral waste applied to land. It is believed that organic trace minerals provide alternative pathways for absorption, thus leading to a reduction in the excretion of minerals (Scott *et al.*, 1982; Leeson, 2003).

However, research into the use of organic trace minerals in broiler chicken diets is still at a nascent stage. Most studies on organic minerals for broilers have used conventional diets, which makes it difficult to separate the effect of the supplemental minerals from that of the native minerals in the ingredients (Henry *et al.*, 1989; Hess *et al.*, 2001; Lee *et al.*, 2001; Paik, 2001; Chowdhury *et al.*, 2004; Revy *et al.*, 2004). In addition, purified diets usually decrease feed intake of broilers and can not support the bird to reach its growth potential, leading to the compromised growth of the chick due to deficiency of other nutrients (Wedekind *et al.*, 1992). The present study was conducted using a semi-conventional control diet, based mainly on sorghum and

isolated soy, to evaluate possible response of broilers to organically complexed Cu, Fe, Mn, Zn on performance, trace mineral excretion and tissue accumulation.

4.2 Materials and methods

The experiment was approved by the Animal Ethics Committee of the University of New England (Approval No: AEC 04/147).

4.2.1 Animal husbandry

During the first two weeks, 160 one-day old Cobb broilers (45.48±1.61 g/bird) were randomly allocated to 40 multi-compartment brooder units located in two temperature-controlled rooms, with eight replicates (four chicks in each cage) per dietary treatment. Each cage contained a water trough and a feeder. Room temperature was maintained at 34°C during the first three days and gradually reduced to 28°C at the end of the second week. Body weight and feed intake were recorded weekly. At 14 days of age, groups of four chicks were individually weighed and transferred to metabolism cages. After four days of adaptation period, all excreta were collected over 4 days and analysed, to evaluate excretion of trace minerals. At 29 d, all birds were killed and blood, liver and right tibia were sampled to analyse for their mineral contents as per Section 3.2.2.

4.2.2 Dietary treatments

The experimental design consisted of 5 treatments with 8 replicate cages per treatment. The dietary treatments were as follows: 1) control diet (Table 4.1) was formulated to either meet or exceed NRC (1994) nutrient requirements with the exception of Cu, Fe, Mn and Zn, which were added to the experimental diets separately (Table 4.2); 2) Organic 1(Low-Org) was the control diet supplemented with 2 mg Cu/kg diet, and 20 mg /kg diet each of Fe, Mn and Zn; 3) Organic 2 (Mid-Org) was the control diet supplemented with 4 mg Cu/kg diet, 40 mg /kg diet each of Fe, Mn, and Zn; 4) Organic 3 (High-Org) was the control diet supplemented with 8 mg Cu/kg diet and 80 mg /kg diet each of Fe, Mn, and Zn; and 5) Inorganic positive control (Inorg) was the control diet supplemented with 5 mg Cu/kg diet, 70 mg Fe/kg diet, 80 mg Mn/kg diet and 50 mg Zn/kg diet in form of sulphate.

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Table 4.1 Composition of the control diet

Ingredient	g/kg
Sorghum	771.00
Isolated soy	175.00
Vegetable oil	16.00
Calcium carbonate	12.46
Calcium phosphate	18.20
NaCl	2.50
Lysine-HCl	1.00
DL-Methionine	2.34
Vitamin/Mineral premix	1.00
Choline Chloride	0.50
Total	1000.00

Nutrient composition

ME (MJ /kg)	13.06
CP (g/kg)	225.0
Ca (g/kg)	8.4
Available P (g/kg)	4.2
Lysine (g/kg)	11.2
Cu (mg/kg ,as fed, analysed)	4.2
Fe (mg/kg, as fed, analysed)	42.2
Mn (mg/kg, as fed, analysed)	14.8
Zn (mg/kg, as fed, analysed)	20.4

Table 4.2 Supplemental levels of minerals fed to birds on different diets (mg/kg)

Diet	Added Cu	Added Fe	Added Mn	Added Zn
Control	0	0	0	0
Low-Org	2	20	20	20
Mid-Org	4	40	40	40
High-Org	8	80	80	80
Inorganic	5	70	80	50

4.2.3 Measurements

Birds were weighed individually at the start, then weekly and at the end of the experiment. Feed intake in each cage was recorded to determine FCR, and both were corrected for mortality.

4.2.4 Excreta collection

At 14 days of age, all chicks were weighed and transferred to metabolism cages. Between 19 and 22 days of age, total excreta from each cage were collected daily and dried at 80°C in a forced draft oven. Fresh and dry weights of faeces were recorded.

4.2.5 Tissue and blood sample collection

Samples of blood and tissue were collected as per Section 3.2.1.

4.2.6 Chemical analysis.

Feed samples were prepared for inductively coupled plasma emission spectroscopy (ICP) (Vista MPX, Melbourne, Australia) by grinding them to pass through a 0.5-mm screen in a stainless blade grinder. After grinding, 0.5 g of samples was placed in a Teflon TFM vessel. Eight mL of nitric acid (70 %) was added along with 2 mL of hydrogen peroxide (30%). The vessel was closed and introduced to the rotor segment, then tightened using a torque wrench. The segment was inserted into the microwave cavity (Ethos Plus Microwave Labstation, Milestone Inc, Monroe, USA) and the temperature sensor connected. The microwave program was run for 45 min. The rotor was cooled by air until the solution reached room temperature. The vessel was opened and the solution quantitatively transferred into a 50 mL volumetric flask. The solution

was made to 50 mL total volume with deionised water and mixed well for ICP analysis (Milestone Application Note 034, 2000).

Faecal samples were prepared for analysis by drying at 80°C for 3 d, and cooling to room temperature. The samples were then ground to pass through a 0.5 mm screen in a stainless steel blade grinder. After grinding, 1 g of samples was ashed at 550° for 4 h (AOAC, 1996). The ash was then dissolved in 10 ml of 3 M hydrochloric acid and boiled for 10 minutes. After cooling, it was diluted to 100 ml with deionised water and the mixture was used to analyze for Cu, Fe, Mn and Zn with ICP (Dozier *et al.*, 2003).

Tibia samples were boiled for approximately 10 min in deionized water and cleaned of all soft tissue. Then the samples were dried for 12 hours at 105°C. Liver samples were thawed and rinsed with deionized water and dried for 12 hours at 105°C. Then liver, bone samples were ashed (550°C for 4h). Approximately 1 g of ash samples was then dissolved in 10 ml of 3M hydrochloric acid and was boiled for 10 minutes. The samples were allowed to cool and filtered into a 100 ml flask. It was diluted to 100 ml with deionized water and analysed for Cu, Fe, Mn and Zn. For measurement of Cu, Fe, Mn and Zn contents in the plasma, 4 ml of plasma sample was wet-ashed in a beaker by adding 10 ml nitric acid and heated to minimal volume (the solution was never allowed to dry). When the solution was cooled, it was filtered into a 25 ml of flask and diluted to 25 ml with deionized water for ICP analysis.

4.2.7 Statistical analysis

Statistical analyses were performed as described in Section 3.3. The data were analyzed using one-way ANOVA with diet as the factor. The significance of difference between means was determined by Duncan's multiple range test. Regression analysis was carried out only with control diet and different levels of organic treatments.

4.3 Results

4.3.1 Broiler performance

During the first week, there was no significant difference in feed intake between the control and experimental groups. After one week, the birds on the control diet started to show symptoms of mineral deficiencies, including reduced feed intake and consequently reduced body growth. Supplemental Cu, Fe, Mn and Zn, regardless of their source, improved (P<0.01) broiler performance (Table 4.3). Adding increased levels of organic trace minerals to the control diet significantly increased body weight gain and had positive effects on FCR (Figure 4.1). The Mid-Org diet achieved a superior (P<0.01) FCR than the inorganic positive control. However, there was no additional response in growth and FCR for the High-Org diet. The control diet had Cu, Fe, Mn and Zn below NRC requirements for broilers. Therefore, the birds on the control diet grew poorly but survived for the entire experimental period. The deficiency of Cu, Fe, Mn and Zn in the control diet strongly affected feed intake, which led to depressed growth of broilers.

Table 4.3 Effect of different diets on broiler chicken performance (0-29 d)

Diet	0-7 d Feed intake (g/bird)	Weight gain (g/bird)	Feed intake (g/bird)	FCR (intake/gain)
Control	147.6	979.9 [c]	1567.5 [b]	1.590±0.093 [a]
Low-Org	146.9	1380.7 [b]	2077.1 [a]	1.510±0.094 [b]
Mid-Org	145.6	1499.5 [a]	2100.3 [a]	1.403±0.067 [c]
High-Org	151.0	1494.5 [a]	2137.4 [a]	1.432±0.052 [bc]
Inorganic	154.6	1481.9 [a]	2210.0 [a]	1.493±0.069 [b]
Pooled SEM	4.95	57.95	37.37	0.027
P value	0.70	<0.001	<0.001	<0.001

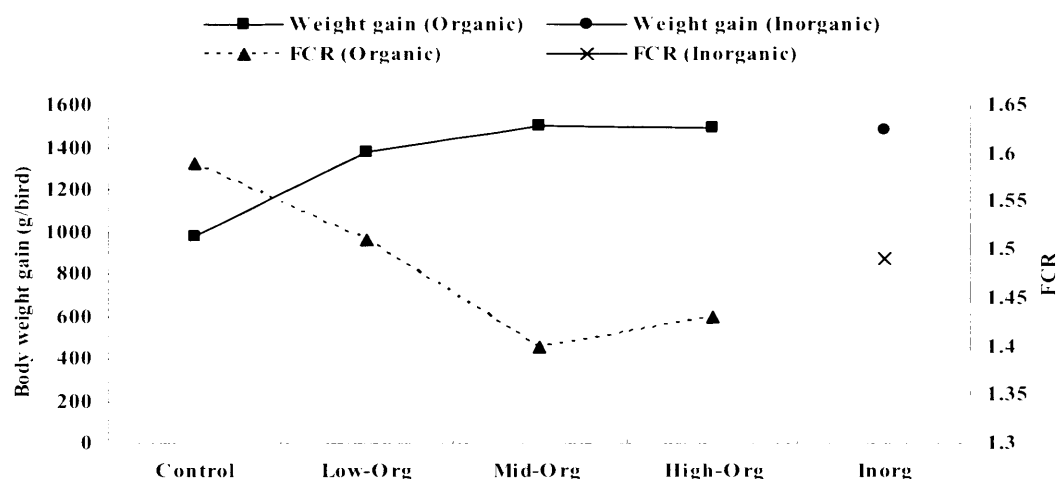[a, b, c] means within a column with unlike superscripts differ significantly (P<0.05).

Figure 4.1 Body weight gain and FCR in response to dietary trace mineral concentrations

4.3.2 Mineral excretion

The excretion of Cu, Mn and Zn increased ($P<0.001$) linearly with increasing intakes of these trace minerals (Table 4.4). Thus the birds on the Mid-Org diet, which supported the best FCR had a lower ($P<0.001$) trace mineral excretion than those on the High-Org treatments.

Table 4.4 Trace mineral excretion in birds fed different diets (mg/bird/d) (18-21d)

Diet	Cu	Fe	Mn	Zn
Control	0.28 [d]	17.66 [b]	1.34 [d]	2.30 [d]
Low-Org	0.56 [c]	17.05 [b]	3.85 [c]	4.44 [c]
Mid-Org	0.83 [b]	24.74 [b]	6.22 [b]	6.47 [b]
High-Org	1.22 [a]	30.27 [ab]	10.12 [a]	10.91 [a]
Inorganic	0.86 [b]	46.66 [a]	8.92 [a]	11.05 [a]
Pooled SEM	0.047	7.143	0.425	0.547
P value	<0.001	0.039	<0.001	<0.001
Regression to intake (R^2)	90.61	7.22	90.28	88.38
P value	<0.001	=0.137	<0.001	<0.001

[a, b, c, d] means within a column with unlike superscripts differ significantly ($P<0.05$).

4.3.3 Mineral concentrations in tibia

Table 4.5 shows that only the concentration of Zn in tibia increased (P<0.001) linearly with Zn intake (Figure 4.2). The Cu and Mn concentrations were also increased (P<0.001) with supplemental dietary Cu and Mn compared with the control diet, but there was no significant difference (P>0.05) between supplemental levels. There was no significant difference (P>0.05) in tibia Fe concentration between treatments.
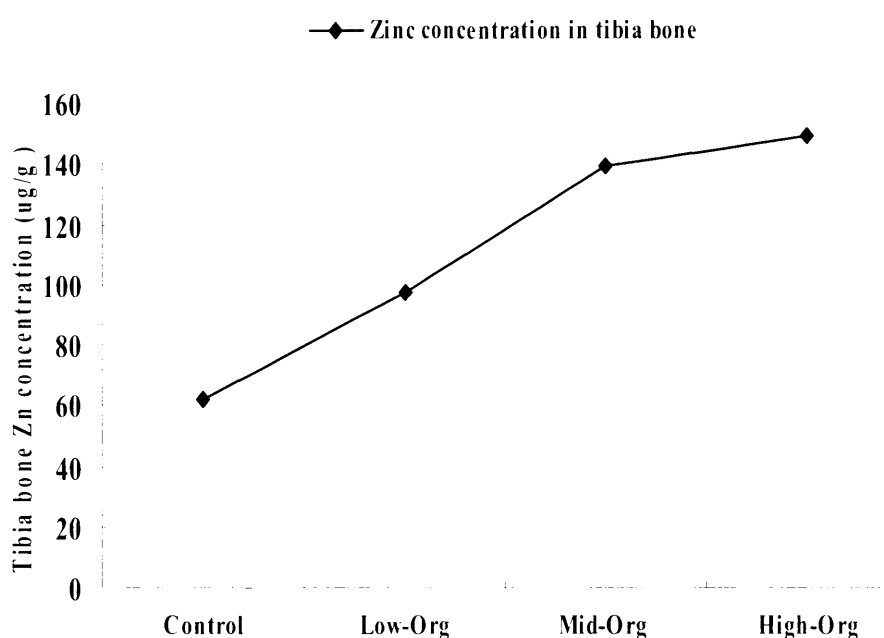

Figure 4.2 Tibia bone Zn concentrations in response to different levels of organic trace minerals at 29 d of age.

4.3.4 Mineral concentrations in liver and plasma

At 29 days of age, the body weight of birds fed the control diet was only 70 % that of birds on supplemental treatments but there was no significant difference (P>0.05) in plasma trace mineral concentrations (Figure 4.3). Trace mineral concentrations in the liver of birds on the control diet were higher (P<0.05) than those on the supplemental treatments but there was no significant difference (P>0.05) among different supplemental treatments (Figure 4.4).

Table 4.5 Trace mineral concentrations of tibia bone of broiler chickens at 29 days of age (μg/g dry bone)

Diet	Cu	Fe	Mn	Zn
Control	2.96 [b]	54.65	2.64 [c]	61.91 [d]
Low-Org	4.81 [a]	69.38	3.61 [b]	97.72 [c]
Mid-Org	5.90 [a]	67.46	3.56 [b]	139.73 [b]
High-Org	5.94 [a]	65.68	4.16 [ab]	148.91 [ab]
Inorganic	6.37 [a]	66.62	4.47 [a]	160.16 [a]
Pooled SEM	0.57	4.55	0.22	5.56
P value	<0.001	0.183	<0.001	<0.001
Regression to intake (R^2)	0.173	0.052	0.39	0.703
Regression to weight gain (R^2)	0.604	0.506	0.242	0.621

Cu (μg/ml)

Fe (μg/ml)

Mn (μg/dl)

Zn (μg/ml)
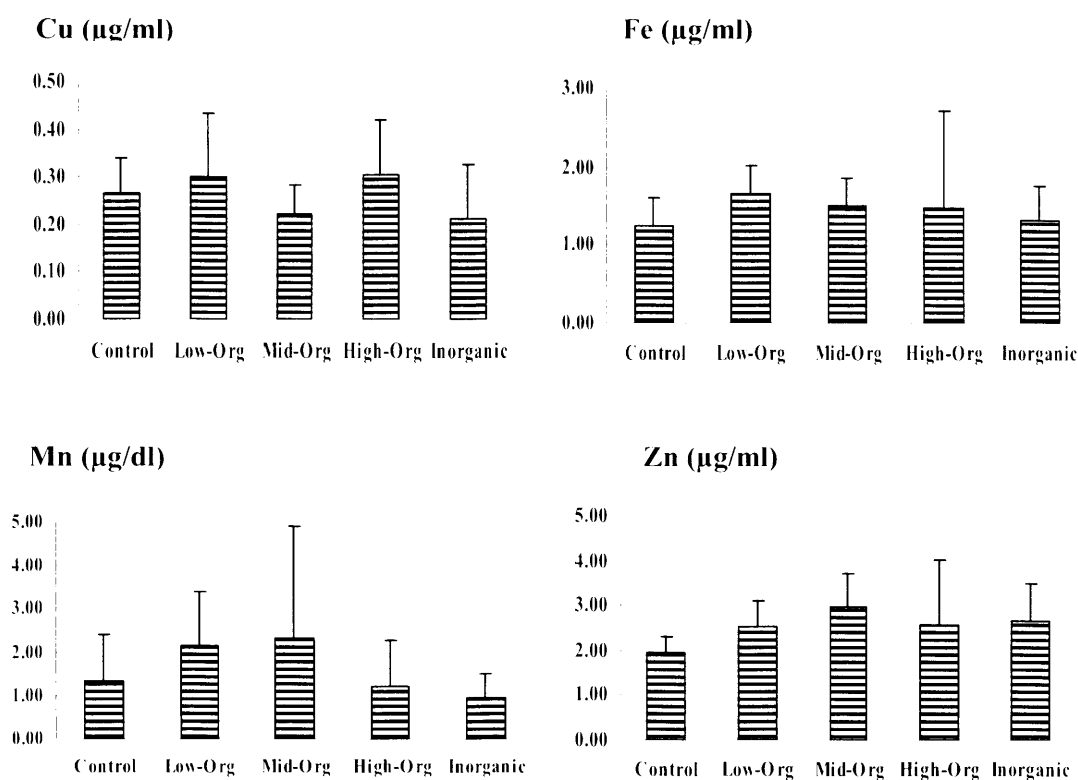
Figure 4.3 Concentrations of Cu, Fe, Mn and Zn in the plasma of chickens on different diets (means±sd).
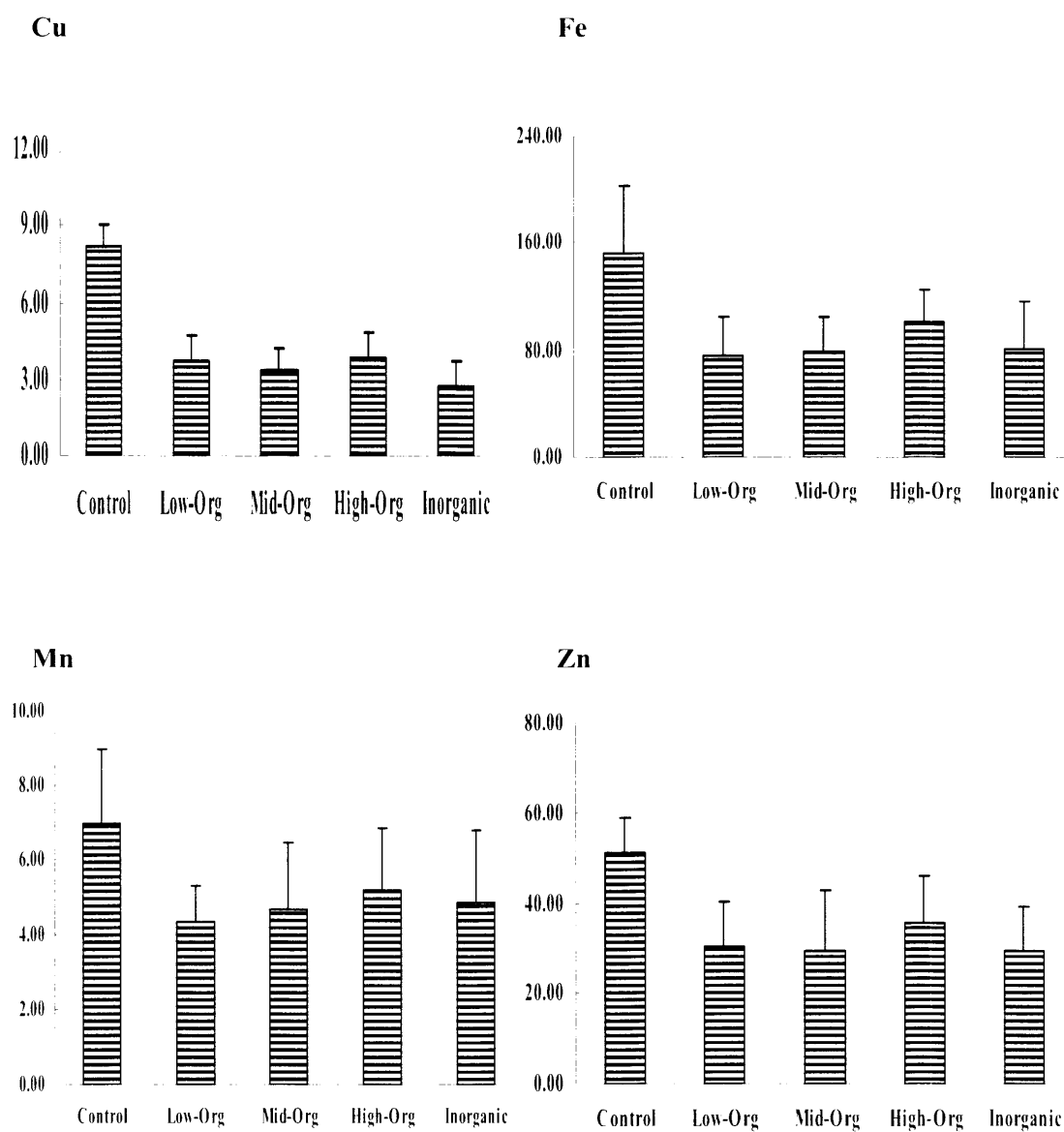
Cu

Fe

Mn

Zn

Figure 4.4 Concentrations (µg/g dry tissue) of Cu, Fe, Mn and Zn in the liver of chickens on different diets (means±sd).

4.4 Discussion

4.4.1 Bird performance

Due to deficiency of Cu, Fe, Mn and Zn in the control diet, feed intake was strongly affected, which led to depressed growth of broilers. This is similar to the symptom of deficiency of zinc described by King *et al.* (1990) that a marked reduction in dietary Zn is invariably followed quickly by a reduction in feed intake and growth failure.

The mechanisms involved in the effects of deficiency of zinc on growth are unknown but a reduction in feed intake may be a protective response to ensure survival and maintain relatively normal, albeit down-regulated metabolic levels of these minerals (MacDonald, 2000). The birds on Mid-Org diet reached optimal body weight gain and were 53 % heavier than the birds fed to control diet. However, High-Org treatment with the highest supplemental levels of the four minerals which was close to the commercial recommendation of the minerals did not show any further response in weight gain and FCR. So it may not be necessary to supplement these organic minerals at levels as high as those of the inorganic supplements. Different from previous reports (Wedekind *et al.*, 1992; Yu *et al.*, 2005), organic treatments did not show any improvement in feed intake, compared with the positive control. In contrast, the birds on Mid-Org diet supported the best FCR due to less feed intake compared with positive control.

4.4.2 Mineral excretion

The results of trace mineral excretion suggests clearly that the highest levels of organic Cu, Mn and Zn tested in the current study do not contribute to bird growth but are excreted. Indeed, it is well known that changes in trace mineral absorption and excretion in the GIT are primary mechanisms for maintaining trace minerals homeostasis (King *et al.*, 2000). Due to this pattern of excretion, the apparent absorption of Cu, Fe, Mn and Zn is not suitable to assess the bioavailability of trace minerals (Ammerman, 1995b). The pronounced reduction in Zn and Cu excretion could only be due to the dietary manipulation of these two trace mineral levels (Mohanna and Nys, 1998; Dozier *et al.*, 2003). The current experiment demonstrated that the highest level of organic trace mineral supplementation had no additional effects on broiler performance and it is possible to use lower levels of organic trace mineral supplements without compromising bird growth or increasing the rate of excretion. It is also strongly suggested that optimal supplemental levels of Cu, Fe, Mn and Zn should not exceed the current High-Org dietary level. In the assessment of trace mineral absorption, the Mid-Org or lower levels of supplementation should be used, since an excess of trace minerals always leads to underestimation of the potential bioavailability (Underwood and Suttle, 1999c).

4.4.3 Mineral concentrations in tibia

It has been observed that when the dietary Zn content was greater than the requirement for growth, there is an increase in the plasma and tibia concentration until a dietary concentration of 48 mg Zn/kg diet was reached (Pimentel et $al.$, 1991a; Mohanna and Nys, 1999). In the current experiment, tibia Zn concentrations were also strongly related to the dietary organic Zn intake ($R^2 = 0.703$) but the tibia Zn concentration reached a plateau as birds attained optimal body weight on the diet in which the dietary Zn concentration was 60 mg/kg diet. The bone is a complex heterogeneous tissue which supports the musculature and, thus, its growth and development are intimately connected with overall body growth (Loveridge, 1992). The optimal Zn for growth is similar to the optimal Zn requirement for maximizing bone concentration, making tibia Zn concentration a good predictor of whole body growth. This result may imply that Zn content in the current control diet is a primary factor that affects bird performance.

4.4.4 Mineral concentrations in liver and plasma

Zinc concentrations in tibia, liver and plasma are usually used as parameters to assess Zn status. However, that chickens give priorities to their mineral requirements for vital functions in compromise of body growth is indicated by the normal concentrations of the minerals in the plasma of the control birds. It is demonstrated that Zn content is low enough to affect broiler performance but not significantly affect plasma Zn concentration, suggesting that plasma Zn concentration is not a particularly sensitive index. Surprisingly the concentrations of the trace minerals were higher in the liver of birds on the control diet than in those on the supplemented diets. Similar findings have been attributed to a diluting effect as a result of rapid growth rate on the adequate diets and poor growth rate on the control diet (Roth, 2003). With Zn, the results are consistent with the model for laboratory animals (King, 1990), which shows that if dietary deficiency of Zn is mild, the animal usually reduces the rate of growth and excretion, to maintain normal tissue concentrations. This response is due to the fact that an animal at a stage of development, at which sensitivity to Zn deficiency is high, stops growing immediately when given a low-Zn diet but it maintains a normal concentration of Zn in its tissues (Golden, 1988b). It indicates that the assessment of trace mineral status is difficult and remains an important tricky

challenge. This result is also in agreement with human Zn research in which there appears to be an adaptation to low Zn intake, associated with a reduction in Zn excretion to conserve this micronutrient and maintain normal plasma Zn concentration (Sullivan *et al.*, 1998).

4.5 Conclusion

The broiler chicken is sensitive to trace mineral deficiency. The control diet based on natural ingredients produced marked trace mineral deficiencies. When the broiler diet is deficient in Cu, Fe, Mn and Zn, birds will mainly decrease their feed intake, resulting in poor growth but maintain a relatively normal plasma trace mineral concentration. It is necessary to supplement trace minerals in broiler diets to allow the modern broiler to reach its genetic potential. The growth of broiler is responsive to the addition of increasing levels of organic minerals as is the mineralization of bone, particularly in the case of Zn.

Due to a high level of excretion of inorganic trace minerals in commercial broiler production, organic Cu, Cu, Fe and Zn may provide an alternative way to supplement trace minerals for the birds. In the current experiment, mid-supplemental levels of organic Cu, Fe, Mn and Zn supported a better FCR than inorganic positive control and it seems that these lower levels of organic trace minerals were adequate to support optimum broiler chicken performance at reasonable rates of excretion.

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CHAPTER 5. Effect of Low Supplemental Levels of Organic Trace Minerals on Broiler Performance and Trace Mineral Digestibility along the Small Intestine

Abstract

An experiment was conducted to evaluate the effects of low supplemental levels of organic and inorganic trace minerals on broiler performance and trace mineral digestibility along the small intestine. Under floor-pen conditions, broilers fed a supplementation of 4, 20, 40 and 30 mg/kg diet respectively of Cu, Fe, Mn and Zn from organic sources achieved the same body weight gain as those supplemented with NRC levels from inorganic salts. However, birds on a control diet without any supplementation at dietary levels of 20 and 15 mg/kg of Zn and Mn, respectively, demonstrated depressed feed intake and growth. Copper might be passively absorbed in all regions along the small intestine. Throughout the small intestine absorption of Mn from both organic and inorganic sources was poor, whereas Zn absorption seemed to be more complex. Organic Zn was absorbed more efficiently (P<0.01) in the ileum. Compared to the ileum, the duodenum tended to have a lower (P<0.05) capability for Zn absorption. Therefore, relatively low levels of supplementation of organic trace minerals were enough to support a normal growth rate and bone development. Organic Zn absorption was improved in the ileum but organic Zn and Mn supplementation is still required to be optimized.

5.1 Introduction

In commercial broiler production, a high safety margin in feed formulation for supplemental inorganic trace minerals has resulted in a high level of mineral excretion, which is harmful to the environment. It is believed that organic trace minerals are better absorbed and utilized than their inorganic counterparts and are protected from interactions that interfere with their bioavailability, thus leading to a reduction in the excretion of minerals (Scott *et al.*, 1982; Leeson, 2003). However, in the first experiment (Chapter 4), the excretion of Cu, Zn and Mn linearly increased

with increasing intake of these organic trace minerals and the pronounced reduction in Zn and Cu excretion could only be achieved on reduced dietary levels of these trace mineral levels (Mohanna and Nys, 1999; Dozier *et al.*, 2003). Therefore, even with organic trace minerals, it is better to use a lower level of supplementation in feed formulation in order not to increase mineral excretion. It was reported that dietary zinc concentration at 45 mg/kg is sufficient to obtain normal broiler growth to 21 d of age but tibia Zn concentration only plateaus at 75 mg/kg from both organic and inorganic sources (Mohanna and Nys, 1999). With 9-day-old broilers, it was demonstrated that there was no difference in body weight and feed intake between those fed a 28 mg Zn/kg maize-soybean basal diet and diets supplemented with high levels of Zn (Cao *et al.*, 2002). Thus it is necessary to investigate the effect of low levels of trace minerals on performance in the entire period of broiler production.

In addition, there are conflicting results about absorption sites for trace minerals (Underwood and Suttle, 1999f) due perhaps to the complexity of trace mineral excretion. It is well known that GIT is a major route of excretion for Cu, Fe, Mn, Zn (Ammerman, 1995b). In the GIT, apart from indigestible trace minerals, the digesta also contains endogenous excretion from cell abrasion and mineral excess which can not be absorbed. This makes it difficult to assess the bioavailability of trace minerals by their apparent absorption. A control diet, which is deficient in Cu, Fe, Mn and Zn, is able to avoid trace mineral excess in the GIT. The determination of absorption sites and digestibility of organic trace minerals in different parts of the digestive tract may provide a pathway to elucidate the mechanism of organic trace mineral absorption.

Therefore, the aim of this study was to evaluate the effects of low levels of organic and inorganic dietary trace minerals on broiler performance, tibia trace mineral concentrations at different ages and the digestibility of trace minerals along the small intestine of 35-day-old broiler chickens reared under floor-pen conditions.

5.2 Materials and methods

The experiment was approved by the Animal Ethics Committee of the University of New England (Approval No: AEC 04/147).

5.2.1 Animal husbandry

A total of 800 day-old male *Cobb* broiler chicks were randomly allocated to 32 deep litter pens with 8 replicates of 25 birds per treatment. In the first two weeks, the birds were given starter diets, followed by the finisher diets for 3 weeks. During the finisher period, Celite (acid insoluble ash) was added to all the diets as a marker for digestibility measurement. All the birds were fed *ad libitum* throughout the experiment. For the first three days, the shed temperature was maintained at 35°C and gradually reduced to 23°C at 28 d of age and maintained till the end of the experiment. Mortality was recorded daily while body weight and feed intake were recorded weekly for the calculation of body weight gain and FCR corrected for mortality. On d 21 and 35, two birds from each pen were randomly selected, weighed and killed by cervical dislocation. Samples of right tibia and digesta from the duodenum, jejunum and ileum were collected and pooled per pen. All samples were frozen at -20°C and later analysed for the trace mineral contents.

5.2.2 Dietary treatment

Four diets were formulated with sorghum and isolated soy as the main ingredients, and pelleted to reduce segregation. Diet 1 acted as the control group (Table 5.1) with all the nutrients either meeting or exceeding the NRC (1994) nutrient requirements except for Cu, Fe, Mn and Zn, which were maintained at 7.4, 60.1, 14.6 and 19.1 mg/kg for the starter diet and 8.8, 69.2, 15.4 and 20.6 mg/kg for the finisher diet. Three other diets were derived from the control diet with variations only in the source and level of Cu, Fe, Mn and Zn contents; as shown in Table 5.2. Diet 2 served as the organic diet (Org) and was supplemented with organic sources of Cu, Fe, Mn, Zn at 4, 20, 40 and 30 mg/kg diet, respectively. Diet 3, inorganic diet (Inorg), was supplemented with corresponding levels of Cu, Fe, Mn and Zn from an inorganic source. Diet 4, the NRC diet (NRC) was similar to Inorg diet except that it was supplemented from a feed-grade sulphate source at 8 mg Cu, 40 mg Fe, 60 mg Mn and 40 mg Zn/kg diet.

Table 5.1 Composition of control diet

g/kg	Stater	Finisher
Sorghum	771.00	819.65
Isolated soy	175.00	120.00
Vegetable oil	16.00	20.00
Calcium carbonate	12.46	11.00
Calcium phosphate	18.20	17.00
Sodium chloride	2.50	2.50
Lysine-HCl	1.00	1.00
Celite	0.00	5.00
DL-Methionine	2.34	2.35
Vitamin/Mineral premix	1.00	1.00
Choline Chloride	0.50	0.50

Nutrient composition

ME (MJ/kg)	13.06	13.25
CP (g/kg)	225.0	185.0
Ca (g/kg)	8.9	8.5
Available P (g/kg)	3.6	3.4
Lysine (g/kg)	13.6	10.2
Cu (mg/kg, as fed, analysed)	7.40	8.80
Fe (mg/kg, as fed, analysed)	60.10	69.2
Mn (mg/kg, as fed, analysed)	14.60	15.4
Zn (mg/kg, as fed, analysed)	19.10	20.6

Table 5.2 Supplemental levels of minerals fed to birds on different diets (mg/kg)

Diet	Added Cu	Added Fe	Added Mn	Added Zn
Control	0	0	0	0
Organic	4	20	40	30
Inorganic	4	20	40	30
NRC	8	40	60	40

5.2.3 Measurement

Birds were weighed in groups at the start, then weekly and at the end of the experiment. Feed intake from each cage was recorded weekly to determine the FCR.

Copper, Fe, Mn and Zn digestibility up to the duodenum, ileum and jejunum were estimated from the analysis of acid insoluble ash (AIA) and mineral concentrations in feed, freeze-dried digesta from these regions using the equation:

$$Trace\ Mineral\ Digestibility = 1 - \frac{\left(\dfrac{Trace\ Minerals}{AIA}\right)_{digest}}{\left(\dfrac{TraceMinerals}{AIA}\right)_{diet}}$$

5.2.4 Chemical analysis

Feed samples were prepared and analyzed as described in Section 4.2.6. Bone ash samples were prepared as per Section 4.2.6 and digesta samples were prepared as per Section 3.2.1 and 3.2.2.

As for the bone ash and digesta samples, approximately 0.2 g of bone ash was placed into a Schott bottle in a scrubbed fume cupboard. Two mL of a mixture of $HClO_4$ (70%) and H_2O_2 (30%) was added to each tube. Each tube was loosely covered with a lid and left overnight. Then 1 mL of H_2O_2 was added and tubes were tightly sealed and placed in an oven set at 80°C for 30 minutes. The bottles were allowed to cool slightly and a further 1 mL H_2O_2 was added before they were capped tightly and digested for 1 hour at 80°C. The solution was made to a weight of 25 g and filtered through Whatman No. 1 filter paper for ICP analysis (Anderson and Henderson, 1986).

The concentration of AIA in the feed and in the freeze-dried digesta was determined using the method described by Vogtmann *et al.*(1975) and Choct and Annison (1990). Briefly, 3 g of diet samples or 1 g of the digesta were weighed accurately into Pyrex[R]-brand Gooch-type crucibles with 4 μm porosity. The samples were dried overnight at 105°C and later ashed overnight at 480°C. After ashing, the crucibles were placed in a boiling 4M HCl acid bath for about 15 minutes and then rinsed with 4 M HCl and distilled water on the vacuuming flasks, and subsequently dried at 105°C for 2 h. The samples were ashed, boiled and rinsed again, and finally dried overnight at 105°C. The AIA was calculated using the equation:

$$AIA\ (g\ /\ kg\ Dry\ matter) = \left(\frac{(Crucible + Ash\ weight) - (Crucible\ weight)}{(Crucible + Dry\ sample\ weight) - (Crucible\ weight)} \right) \times 1000$$

5.2.5 Statistical analysis

Statistical analyses were performed as described in Section 3.3. The data were analysed using one-way ANOVA with diet as the factor. The significance of difference between means was determined by Duncan's multiple range test.

5.3 Results

5.3.1 Broiler performance

Bird performance results are shown in Table 5.3. During the first week, birds on the control diet had already shown reduction ($P<0.001$) in both feed intake and body weight gain. This trend was maintained throughout the 35 days of growth and became more pronounced towards the end of the trial. However, there was no significant difference ($P>0.05$) in FCR between the control diet and the supplemental diets during the periods of 14-35 d and 1-35 d. During the periods of 1-14 d and 1-35 d, there was no significant difference ($P>0.05$) in mortality rates between treatments, although the birds fed the control diet showed much smaller body weight gain. However, during the d 14-35, there were significant differences in mortality rates between control diet and supplemental treatments ($P<0.05$).

Table 5.3 Bird performance on different diets

Period	Control	Organic	Inorganic	NRC	Pooled SEM	P value
Feed Intake (g/bird)						
1-7 d	123 [c]	170 [ab]	156 [b]	186 [a]	7.9	<0.001
1-14 d	315 [b]	514 [a]	521 [a]	521 [a]	13.1	<0.001
14-35 d	1283 [b]	2623 [a]	2616 [a]	2590 [a]	58.9	<0.001
1-35 d	1597 [b]	3137 [a]	3137 [a]	3111 [a]	62.4	<0.001
Body weight gain (g/bird)						
1-7 d	103 [b]	151 [a]	153 [a]	151 [a]	3.2	<0.001
1-14 d	239 [b]	426 [a]	430 [a]	427 [a]	6.9	<0.001
14-35 d	782 [b]	1587 [a]	1607 [a]	1524 [a]	37.0	<0.001
1-35 d	1020 [b]	2013 [a]	2038 [a]	1950 [a]	40.9	<0.001
FCR						
1-7 d	1.20 [a]	1.13 [ab]	1.03 [bc]	1.23 [a]	0.042	0.010
1-14 d	1.32 [a]	1.21 [b]	1.21 [b]	1.22 [b]	0.031	0.042
14-35 d	1.64	1.71	1.66	1.63	0.049	0.677
1-35 d	1.57	1.57	1.54	1.60	0.037	0.741
Mortality (%)						
1-7 d	0.5	1.5	0.5	3.0	0.81	0.117
1-14 d	1.5	2.5	1.0	4.0	1.17	0.303
14-35 d	10.5 [a]	5.6 [b]	6.1 [b]	6.3 [b]	1.08	0.012
1-35 d	12.5	8.0	7.5	10.5	1.88	0.231

Data represent means of eight replicate groups of 25 chickens during period 1 to 35 d post-hatching.

[a,b] means within the same row with no common superscript differ significantly (P<0.05).

5.3.2 Mineral concentrations and ash weight in tibia

Table 5.4 shows that there were no significant differences (P>0.05) among the treatment groups in tibial concentrations of both Cu and Fe. However, dietary deficiency in trace minerals strongly affected (P<0.001) Mn and Zn concentrations and total ash weight in the tibia (P<0.001) of both 21 and 35-day-old broiler chickens but there was no significant difference (P>0.05) between supplemental treatments. Dietary zinc concentration of 50 mg/kg from both the inorganic and organic sources resulted not only in normal growth but also showed a plateau in tibial zinc

concentration. Apart from Zn and Mn, there was no significant difference in Ca, P, Cu and Fe concentrations in tibia between the control diet and the supplemental diets.

Table 5.4 Mineral concentrations in tibia bone (μg/g)

Parameters	Control	Organic	Inorganic	NRC	Pooled SEM	P value
At 21 d						
TTA[1]	0.34[b]	0.64[a]	0.67[a]	0.68[a]	0.02	<0.001
Ca (%)	14.0	15.2	15.9	16.2	0.61	0.648
P (%)	6.5	7.1	7.4	7.2	0.17	0.007
Cu	1.8	1.6	1.7	1.6	0.29	0.832
Fe	65.5	65.0	72.6	82.3	1.82	0.167
Mn	1.1[b]	2.9[a]	2.8[a]	2.9[a]	0.17	<0.001
Zn	73.8[b]	182.0[a]	173.5[a]	181.0[a]	7.16	<0.001
At 35 d						
Ca (%)	13.4	13.8	13.7	13.8	0.40	0.913
P (%)	6.3	6.5	6.3	6.4	0.16	0.867
Cu	1.6	1.4	1.4	1.5	0.08	0.277
Fe	81.4	77.0	79.6	71.0	6.41	0.685
Mn	1.8[b]	3.2[a]	3.9[a]	3.4[a]	0.20	<0.001
Zn	109.0[b]	146.7[a]	147.5[a]	139.5[a]	4.50	<0.001

1. TTA - total tibia ash; μg/bird

Data represent means of eight replicates of 2 chicken right tibia bones at age of 21 d and 35 d.

[a, b] means within the same row with no common superscript differ significantly (P< 0.001).

5.3.3 Mineral digestibility along the small intestine

As shown in Figure 5.1, the absorption of Cu was stable along the small intestine with no significant difference (P>0.05) in its digestibility between treatment groups or between different regions of the small intestine.
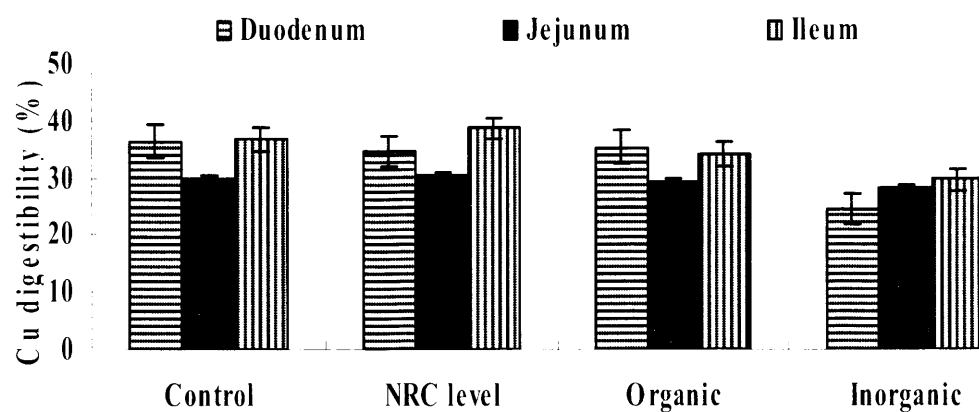

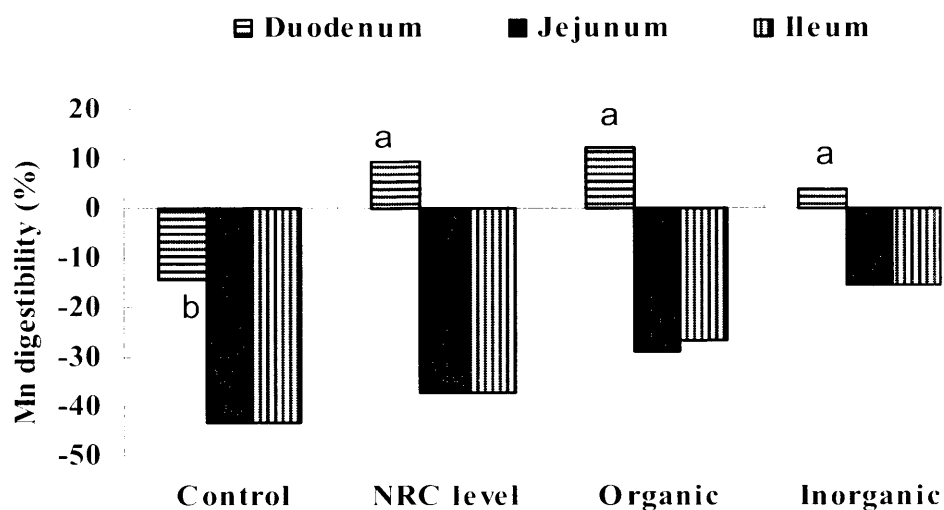Figure 5.1 Cu digestibility in different segments of small intestine on different diets

Figure 5.2 Mn digestibility in different regions of small intestine on different diets

a. b means in different columns with no common superscript differ significantly (P 0.05).

The birds fed the control diet showed poor digestibility of Mn throughout the small intestine and supplementation significantly (P<0.05) increased its digestibility only in the duodenum, but there was no significant difference (P>0.05) between organic and inorganic sources (Figure 5.2).

Zinc was absorbed mainly in the ileum and its digestibility in the ileum and duodenum reached 50-60 and 20%, respectively (Table 5.5). However, dietary supplementation of Zn did not improve (P>0.05) its digestibility up to the jejunum but significantly improved (P<0.001) duodenal Zn digestibility. In the ileum, Zn digestibility of the control group was higher (P<0.001) than that of the inorganic group but similar (P>0.05) to that in the organic group (Table 5.5, Figure 5.3).

Table 5.5 Zn digestibility in different regions of the small intestine (%)

Segments	Control	Organic	Inorganic	NRC	Pooled SEM	P value
Duodenum	-13.7 [b]	33.7 [a]	26.2 [a]	19.2 [a]	4.60	<0.001
Jejunum	-41.8	-0.5	-11.8	-15.1	17.10	0.392
Ileum	63.8 [a]	63.7 [a]	56.2 [b]	55.2 [b]	1.80	0.002

Data represent means of six replicates of two chickens at 35 d post-hatching.

[a, b] means within the same row with no common superscripts differ significantly (P<0.05).
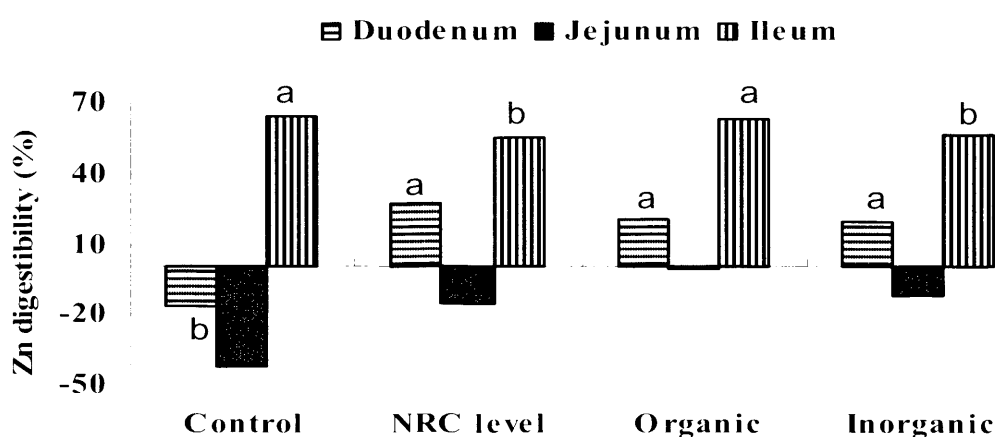
☐ Duodenum ■ Jejunum ⊞ Ileum

Figure 5.3 Zn digestibility in different regions of the small intestine of broilers fed different diets

The value of Fe digestibility within the groups varied widely (30% vs. -0.5%), making it impossible to pool the data for meaningful analysis.

5.4 Discussion

5.4.1 Broiler performance

It is reported that with a basal diet containing 16 mg Cu and 30 mg Zn/kg diet, there was no difference in growth rate or FCR compared with supplemental Zn treatments from placement to 21 d of age (Burrell *et al.*, 2004). On floor-pen conditions, with a basal diet containing 7 mg Cu, 250 mg Fe, 22 mg Mn and 30 mg Zn/kg diet, bird performance was not affected by the levels of supplemental trace minerals (Shelton and Southern, 2006). However, in the current experiment, a control diet containing 7.4 mg Cu, 60 mg Fe, 14.6 mg Mn and 20 mg Zn/kg, which was lower than the NRC recommendations, strongly depressed feed intake from the first week of age. This indicates a primary response to deficiency of trace minerals, especially Zn. This is consistent with the effects of dietary Zn deficiency in rats (Shay and Mangian, 2000), where a reduced amount of Zn-adequate diet, equivalent to the amount consumed by similar Zn-deficient rats was provided, pair-fed control rats experienced depression in growth in an essentially similar fashion to that of the zinc-deficient rats. Compared with the previous results, 30 mg/kg dietary Zn might be a minimal requirement to support a normal growth for broilers. It can be deduced here that the symptoms of trace mineral deficiency as observed in this experiment were probably mainly due to the dietary Zn deficiency. The distinguishing effects among these four elements are further explained in Chapter 7.

Different from the cage experiment in Chapter 4, feed intake of birds fed the control diet started to decline even in the first week of age. The exact reason is unknown. It would appear that birds at early age in deep litter floor pen conditions had less access to trace minerals from other sources except diets and are more sensitive to Zn or other trace mineral deficiency.

It is noticed that birds fed the control diet had a higher mortality rate during d 14-35 compared with those fed supplemental treatments, suggesting that a bigger body weight in fast growing period would accentuate requirement for Zn or other trace

minerals. Reduced feed intake due to trace mineral deficiency could of course have affected survivability of birds. Surprisingly during the entire period of the current experiment, there were no significant differences in body weight gain and feed intake between supplemental treatments, indicating that under floor pen conditions, the current low levels of supplementation of trace minerals might be enough to support normal growth rate for broiler chickens.

5.4.2 Mineral concentrations and ash weight in tibia

The results of mineral concentrations and ash weight in tibia bones demonstrated that Cu and Fe contents in the control diet were sufficient to support normal bone growth when birds maintained a normal feed intake. There was no further effect from additional supplementation. It has been reported that the breakpoint for Zn supplementation on tibia Zn concentration occurred between 54 and 60 mg/kg diet with organic and inorganic supplements, respectively (Wedekind et al., 1992). In the cage experiment (Chapter 4) tibia Zn did not plateau until about 60 mg/kg total Zn supplementation. However, in the current experiment under floor-pen conditions, due probably to a relative increase in nutrient recycling, the dietary Zn concentration of 50 mg/kg from both inorganic and organic sources resulted in normal broiler chicken performance and there was no further effect on tibia Zn concentration with high levels of supplementation. This level of Zn supplementation was close to that reported by Pimental et al. (1991a) and slightly higher than the NRC recommendation (NRC, 1994), but it was lower than the plateau level of 75 mg/kg diet reported by Mohanna and Nys (1999).

Hence, it can be deduced that dietary levels of 12, 80, 55 and 50 mg of Cu, Fe, Mn and Zn per kg, respectively, from organic sources, already meet the requirements of these minerals by broiler chickens up to 35 d of age. Significantly lower concentrations of Zn and Mn in tibia for birds fed on the control diet were due mainly to lower feed intake and thus, poorer growth rate. It was reported that a dietary Zn concentration of 30 mg/kg could support optimal growth for broiler chickens fed a purified diet (Wedekind et al., 1992) and a corn-soybean diet (Cao et al., 2002; Shelton and Southern, 2006). However, the current level of 20 mg Zn per kg diet in the control group tested in this study was obviously insufficient to support normal

growth. Thus, the optimal dietary organic Zn concentration might be in the range of 30 to 50 mg/kg.

5.4.3 Mineral digestibility along the small intestine

There is no satisfactory method to measure the absorption and digestion of trace minerals due to the complexity of endogenous trace mineral excretion in animals. Using lower levels of trace mineral supplementation than usually used in practice, the current experimental results demonstrated that Cu was absorbed in all parts of the small intestine. The control diet and a low level of dietary supplementation of Cu had no obvious effect on the digestibility of Cu. This finding differed from that observed in rats where Cu was primarily absorbed in the duodenum (Linde, 1996). The current results may indicate that chickens regulate their Cu homeostasis via excretion but absorb Cu passively. Thus both organic and inorganic sources of Cu are absorbed efficiently throughout the small intestine.

Manganese was also thought to be absorbed throughout the length of the small intestine (Keen and Zidenberg-Cherr, 1996). It is believed that the Mn requirement of poultry is higher than that of mammals because of its relatively inefficient intestinal absorption (Collins and Moran, 1999). However, the current findings showed that Mn might be absorbed mainly in the duodenum and the absorption rate was very low, suggesting that Mn absorption was probably dependent on that of other trace minerals or growth rate but not by its dietary concentrations. In contrast to Cu, chickens may regulate their Mn homeostasis via changes in absorption and not by excretion.

Usually most of the Fe in excreta represents unabsorbed dietary Fe (Scott et al., 1982). This suggests that birds regulate Fe homeostasis via its absorption. It was not possible to assess Fe absorption in the current study due to large variation in measurements, indicating that Fe absorption might be affected by other unknown factors. In addition, in the control diet, Fe contents might already have exceeded the requirement, skewing its absorption results.

The current results showed that Zn was absorbed mainly in the ileum and duodenum. The digestibility of Zn in the ileum and duodenum reached 50-60% and 20%,

respectively, but its absorption in the jejunum was poor. This is close to the results obtained in the rat where Zn absorption was much higher in the ileum than in the duodenum and jejunum (Lönnerdal, 1989). In the current experiment, Zn absorption from the jejunum was poor, suggesting that this region may be unimportant to Zn absorption for broiler chickens. It was reported that dietary Zn deficiency resulted in morphological and functional changes in the rat jejunum, including shortening and narrowing of jejunal villi (Southon *et al.*, 1985). However, it was clear that in the current experiment, dietary supplementation of Zn did not improve jejunal Zn digestibility. Contrary to the reports on humans and rats where decreased dietary Zn intake always results in increased efficiency in Zn absorption (King *et al.*, 2000), birds on the control diet showed much lower duodenal Zn digestibility than the supplemental groups. In the ileum, it was true that Zn digestibility of the control group was higher than that of the inorganic group but similar to that observed in the organic group due possibly to organic nature of the endogenous Zn content of the diet. It is worth mentioning that as suggested by Lönnerdal (1989), the mechanisms involved in intestinal absorption of Zn can be complicated by the fact that although the ileum might have a higher capacity in absorbing Zn, it is the duodenum that has the first opportunity to absorb the element from the digesta. This would leave little to be absorbed in the ileum. Although, compared to the ileum, the duodenum may have a relatively low capacity to absorb Zn, it has a higher sensitivity towards changes in dietary Zn level. As clearly shown in this experiment, dietary Zn deficiency strongly suppressed its digestibility in the duodenum although there was no significant difference in duodenal Zn digestibility between inorganic and organic groups. The complexity of Zn absorption results indicates that both organic and inorganic Zn were absorbed efficiently in the duodenum, which might be essential to regulate feed intake, and therefore body weight gain. Improved Zn absorption in the ileum for organic Zn and control diet might indicate that broiler chickens regulate its Zn homeostasis via both its excretion and changes in its absorption.

It is known that Cu, Fe, Mn and Zn are susceptible to hydroxy-polymerization and are acid-soluble (Powell *et al.*, 1999). In the peri-neutral intestinal environment, they readily form insoluble hydroxide precipitates. Therefore, it is most likely that the absorption of these trace minerals is regulated in the gizzard by gastric acid output. The gastric acid output might be determined primarily by dietary Zn concentration, in

its regulation of feed intake. The difference in ileal Zn absorption between inorganic and organic treatments or the control diet might primarily be due to different endogenous Zn excretion in the ileum.

Surprisingly such reduced endogenous Zn excretion may not confer any advantage in bird performance over inorganic Zn as shown in the current experiment. The possible reason is that under floor pen conditions, especially in the later period of growth, birds had more access to recycled nutrients, including trace minerals from droppings, reducing the efficacy of organic Zn. In addition, the current low level of inorganic Zn supplementation is already adequate to support a normal growth rate and there was no further space for organic Zn to demonstrate its advantage.

5.5 Conclusion

Under floor-pen conditions, lower supplemental levels of organic Cu, Fe, Mn and Zn than those used in practice were adequate to support normal growth rate and bone development, indicating that these lower levels of organic trace minerals have already met the requirements of these minerals by broiler chickens. Trace mineral absorption is complex. Copper absorption was stable and it was absorbed in all parts of the small intestine, whereas Mn absorption was poor throughout the small intestine and the main absorption site appears to be the duodenum. Supplemental Zn, on the other hand, was efficiently absorbed in the duodenum but not in the jejunum. It was observed that the absorption of organic Zn might be improved in the ileum, which may be due to reduced endogenous excretion compared with inorganic salts.