CHAPTER II

BACKGROUND INFORMATION

Chemical Properties of Zinc

Zinc is a transition metal and a member of group IIb of the periodic arrangement of elements (Burger and Rivers, 1989; O'Dell et al., 1979). The chemically combined form is always that of a divalent cation, with an atomic number of 30 and atomic weight of 65.37 (McDowell, 2003).

Absorption of Zinc

The main site of Zn absorption in the GIT is the proximal small intestine. Zinc is absorbed into the enterocyte by a carrier-mediated process. In addition to carrier-mediated transport, passive diffusion (nonsaturable) and/or paracellular Zn absorption also are thought to occur with high Zn intake (Figure 1). However, the form in which Zn is absorbed into the enterocyte is uncertain. Zinc may move across the brush border as an ion or as part of a chelated complex. Most evidence supports the absorption of Zn as a complex with ligands derived from both exogenous and endogenous sources (Gropper et al., 2005). Transfer of Zn out of the intestinal mucosal cells to the plasma is controlled closely by metallothionein (Pond et al., 1995).

Factors Influencing Zinc Absorption

Absorption of Zn depends on several factors including body Zn status, physiological state, age, health, genetic of the animal, and composition of diet (Wilaison, 2002). Several endogenous substances are thought to serve as ligands of Zn^{2+} which enhance Zn absorption. Possible endogenous ligands include citric acid and picolinic acid. Amino acid ligands include histidine, cysteine, and possibly other amino acids (lysine and glycine). In addition, glutathione (a tripeptide composed of

cysteine, glutamate, and glycine) or products of protein digestion, such as tripeptides, are purported to serve as ligands. Many compounds in food may complex with Zn^{2+} and inhibit its absorption, for example, phytate (Figure 2) that found in plant foods particularly cereals such as maize and bran and legumes can have negative effect on Zn absorption. It binds to Zn via oxygen forming the Zn-phytate complex which is large, insoluble, and poorly absorbed. Other inhibitor of Zn absorption are oxalate (Figure 2), polyphenols, and fibers. Furthermore, other divalent cations (Fe^{2+}, Ca^{2+}, Cu^{2+}) may inhibit Zn absorption probably related to the fact that these cations compete with one another for binding ligands in the intestinal lumen or within the cell as well as for receptor sites on the brush border of the enterocytes (Gropper et al., 2005).

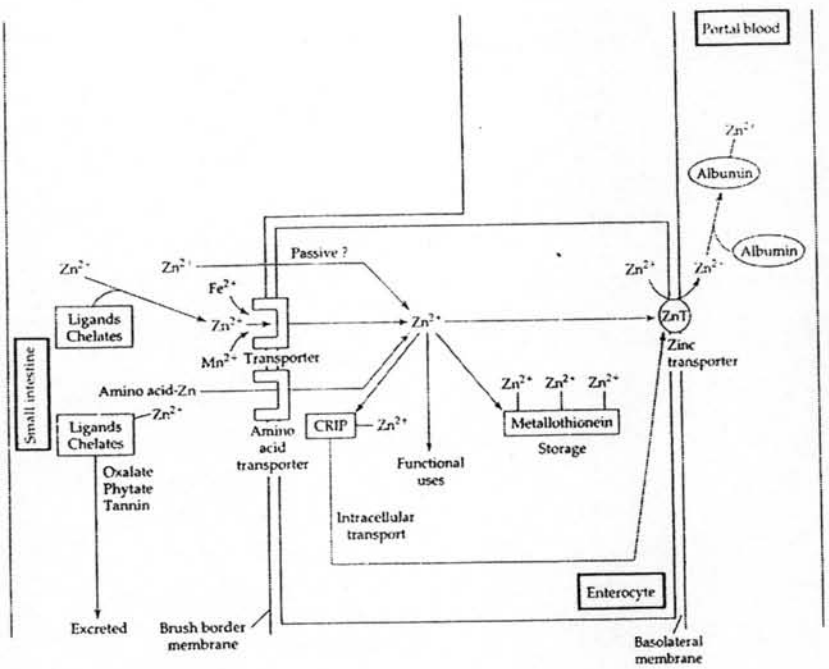
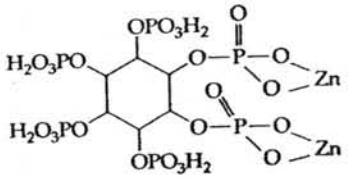
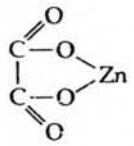


Figure 1. The absorption and transport of Zn into circulation (Gropper et al., 2005)



(a) Phytate



(b) Oxalate

Figure 2. The binding of Zn by phytate and oxalate (Gropper et al., 2005)

Moreover, water solubility of dietary Zn source affected on Zn absorption. Insoluble dietary Zn source will not be absorbed and will pass through the GIT of the animal. Because it cannot dissociate to Zn^{2+} for binding ligands which enhance Zn absorption (Scrimgeour III, 2004). Zinc oxide is relatively insoluble in water. Zinc sulfate and Zn chloride are readily soluble in water, providing ionic Zn. However, they irritate gastrointestinal membranes (Zinpro, n.d.).

Organic Minerals

Hellman and Carlson (2003) described the simply means of organic mineral that the mineral is bound to an organic material. These materials are generally amino acid, protein, polysaccharide, and propionic acid. Scrimgeour III (2004) suggested that organic minerals with different chemical structures also with different chemical properties. Definitions of various types of organic minerals, as provided by the Association of American Feed Control Officials (AAFCO, 2000), are as follows:

1. A metal (specific amino acid) complex is the product resulting from complexing a soluble metal salt with a specific amino acid. Minimum metal content must be declared. When used as a commercial feed ingredient, it must be declared as a specific metal, specific amino complex: i.e., zinc lysine and zinc methionine.

2. A metal amino acid complex is the product resulting from complexing a soluble metal salt with an amino acid(s). Minimum metal content must be declared. When used as a commercial feed ingredient, it must be declared as a specific metal amino complex: i.e., copper amino acid complex and zinc amino acid complex.

3. A metal amino acid chelate is the product resulting from the reaction of a metal ion from a soluble metal salt with amino acids with a mole ratio of one mole of metal to one to three (preferably two) moles of amino acid to form coordinate covalent bonds. The average weight of the hydrolyzed amino acids must be approximately 150 and the resulting molecular weight must not exceed 800. The minimum metal content must be declared. When used as a commercial feed ingredient, it must be declared as a specific metal amino acid chelate: i.e., calcium amino acid chelate, zinc amino acid chelate, and iron amino acid chelate.

4. A metal proteinate is the product resulting from the chelation of a soluble salt with amino acids and/or partially hydrolyzed protein. It must be declared as a specific metal proteinate: i.e., copper proteinate and zinc proteinate.

5. A metal polysaccharide complex is the product resulting from complexing of a soluble salt with a polysaccharide solution declared as an ingredient as the specific metal complex: i.e., copper polysaccharide complex, zinc posaccharide complex, and manganese polysaccharide complex.

Furthermore, other organic minerals that AAFCO has not given the definition including a metal polysaccharide complex and organic metal salt. A metal polysaccharide complex is more of an organic mineral matrix without any chemical bond between the metal and the polysaccharide. This form can be digested by pancreatic enzyme then metal ions bound ligands to be absorbed (Qualitech, n. d.). Scrimgeour III (2004) gave a definition of organic metal salt as one metal ion bonded to propionate or acetate anions, for example, Zn propionate and Zn acetate. This organic metal salt has the chemical property of high solubility, but also readily dissociate in solution. It acts very similar to inorganic sulfate minerals forms. Once in solution the metal is released and must search for a ligand to be absorbed. They do not provide protection from antagonists in the digestive system.

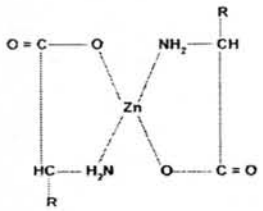
The development and marketing of organic trace minerals has centered around the theory that they are more bioavailable, or more similar than inorganic sources to forms that occur in the body. If the metal chelate or complex is stable in the digestive tract, the metal would be protected from forming complexes with other

dietary components that inhibit absorption and thus allow for greater absorption. Trace minerals are present in the body and function almost entirely as organic complexes or chelates and not as free inorganic ions (Spears, 1996).

Chelated Minerals

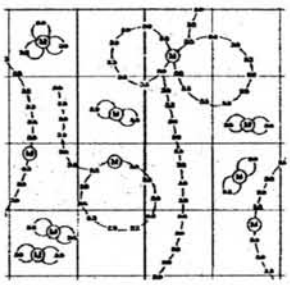
1. Meaning and Classification of Chelated Minerals

The word "chelate" is taken from the Greek word, "chele" meaning "claw" which is a good descriptive term for the manner in which polyvalent cations are held by the metal binding agents. A metal chelate is formed as a ring structure produced by attraction between the positive charges of certain polyvalent cations and any two or more sites of high electronegativity in a chemical compound. The bonds are known as "coordinate" bonds (Scott et al., 1976). The metal ion occupies the central position within the chelate molecule (Ashmead et al., 1985). Thus, by AAFCO (2000) metal amino acid chelate, metal (specific amino acid) complex, metal amino acid complex, and metal proteinate are classified in chelated mineral (Figure 3).



(a) Zn amino acid chelate

(b) Zn (specific amino acid) complex



(c) Zn proteinate

Figure 3. Structures of Zn amino acid chelate, Zn (specific amino acid) complex and Zn proteinate (Albion, 1995, 2000; Zinpro, 2005)

2. Advantages of Chelated Minerals

Vandergrift (1994) summarized the advantages of mineral chelate are as follows: 1) stable in the wide pH range encountered within the different segments of the digestive tract and so it does not dissociate before reach the absorption site; 2) highly soluble in order to improve absorption of the mineral; 3) it does not interact with other substances in GIT because electrically neutral; and 4) amino acid which is bound by mineral acts as a carrier is used to transport that mineral across the intestinal enterocyte and into the circulation.

3. Absorption and Transportation of Mineral Chelate

Lowe et al. (1994a) found that form of Zn has an effect on either the rate or control of Zn transport from the intestinal lumen to the circulation, or on the subsequent clearance from the circulation. Ashmead et al. (1985) reported that in the beginning absorption of the mineral chelate was retarded because it had to move through the intestine past the ionic absorption sites to the peptide absorption sites. The absorption site of the chelate is further down the intestine past the ionic site. Moreover, chelate can be absorbed more than inorganic mineral at the same time as shown in Figure 4. Zinc amino acid chelate (ZnAAC) has greater potential for absorption than inorganic Zn and absorption of ZnAAC is closer to that determined for amino acids (Lowe et al., 1994a). This supports the conclusions of Ashmead (1992) that ZnAAC is transported across the intestinal enterocyte and into the circulation as an intact molecule. In this form the mineral is absorbed not as an ion but as part of a peptide molecule because it occupies a central position between the two amino acids which together with the metal form the chelate. The amino acids "smuggle" the metal into the mucosal cell (Figure 5).

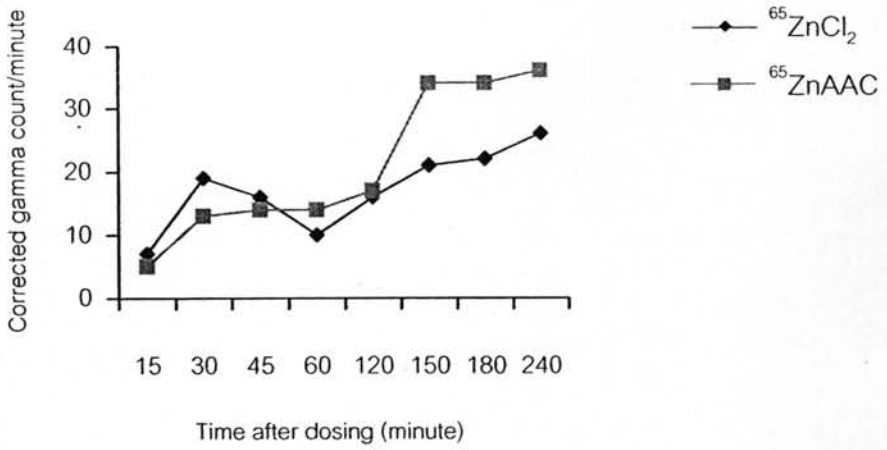


Figure 4. Mean intestinal absorption of zinc chloride versus zinc amino acid chelate as measured in blood of rats (Ashmead et al., 1985)

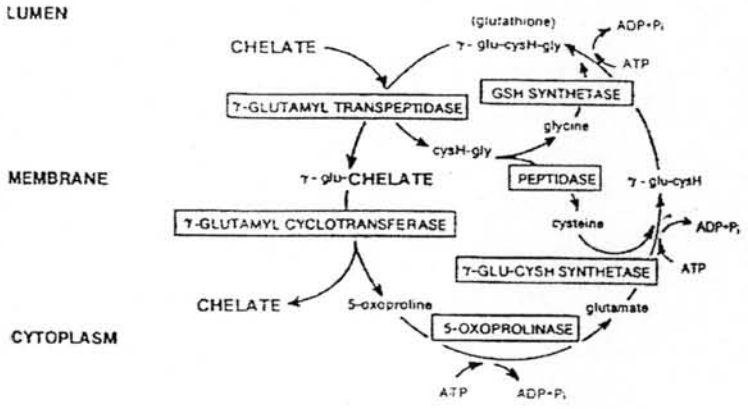


Figure 5. The membrane transport of an amino acid chelate demonstrating the biochemical transport pathway of an amino acid chelate through the cellular membrane (Ashmead, 1992)

As shown in Figure 5, the glutathione is thought to be attached to the amino acid chelate at the terminal amino group (NH_2). This terminal amino group of the chelate is believed to be attached to the γ-glutamyl linkage of the tripeptide in the mucosal cell membrane. The resulting chelate-glutathione compound would then be degraded to γ-glutamyl amino acid chelate and cysteinyl-glycine by the enzyme γ-glutamyl transpeptidase. The next step would be the cleavage of the γ-glutamyl

amino acid chelate by the enzyme γ-glutamyl cyclotransferase. This would degraded the γ-glutamyl amino acid chelate into 5-oxy-L-proline and the metal amino acid chelate. This metal amino acid chelate is moved directly into the plasma as an intact molecule. The involvement of ATP would be necessary to restore glutathione to carry the next molecule of the amino acid chelate (the substrate) across the brush border into the cell. Albion (2004) reported that Zn chelate is absorbed and moved directly into the plasma as an intact molecule. It is metabolized at the target tissue.

On the other hand, the response of Zn concentration in plasma or serum shown that young pigs fed Zn-methionine (ZnMet) had higher ($P < 0.05$) Zn concentration in plasma (Hahn and Baker, 1993) or serum (Hall et al., 1993) than pigs fed ZnO or $ZnSO_4$. Ahn et al. (1998, 1999) reported that weanling pigs fed Zn methionine chelate had greater ($P < 0.01$) serum Zn concentration than pigs fed ZnO. Using Zn proteinate supplemented in nursery pig diets at lower level than ZnO supplemented resulting in no different ($P > 0.05$) in plasma Zn concentration (Carlson et al., 2004). Wilaison (2002) found that increase serum Zn concentration depended on the dietary Zn level. Starter, growing, and finishing pigs fed the same level of Zn, Zn proteinate provided higher ($P < 0.05$) serum Zn concentration than $ZnSO_4$. In addition, Lowe and Wiseman (1998) studied in adult dogs found that ZnAAC had higher ($P < 0.05$) relative bioavailability (RB) of hair growth, Zn deposited in hair, and area under the plasma Zn curve (109, 152, and 129%, respectively) than ZnO. A valid comparison of the RB of Zn from ZnAAC can be made with the standard ZnO (100% RB). In pigs, ZnMet or Zn-lysine had higher bioavailability than ZnO or $ZnSO_4$ (Rupic et al., 1997; Schell and Kornegay, 1996).

Use of Zinc by Intestinal Cell

As indicated in Figure 1, Zn entering the enterocyte has several possible fates. Zinc may be either used functionally within the enterocyte, stored bound to the proteins in the enterocyte, or transported through the cell and across the basolateral membrane into the plasma for use by other tissues (Gropper et al., 2005).

Transportation and Tissue Uptake of Zinc Ion

Zinc ion passing into portal blood from the intestinal cell is mainly transported loosely bound to albumin (Figure 1). From the enterocyte, most Zn is taken up by the liver. Upon departing from the liver, albumin as well as other compounds, such as transferrin, α-2 macroglobulin, and immunoglobulin (Ig) G, bind and transport Zn in the blood. Albumin is thought to transport up to approximately 60% of Zn. Transferrin, α-2 macroglobulin, and IgG are thought to transport approximately 15 to 40% of Zn in the blood. Two amino acids, histidine and cysteine, loosely bind approximately 2 to 8% of the Zn transport; these amino acids form a ternary (histidine-Zn-cysteine) complex in the blood (Gropper et al., 2005).

McDowell (2003) reported that Zn ion is moved into and out of cells by a series of transport proteins or transporters (ZnT). Four members of the family of mammalian ZnT have been characterized, i.e., ZnT-1, ZnT-2, ZnT-3 and ZnT-4. ZnT-1 is expressed in a variety of tissues, including intestine, kidney and liver. Intestinal expression is regional, being much greater in duodenum and jejunum and in villus versus crypt cells. It is not expressed in the globlet cells and lamina propria of small intestine. ZnT-1 expression is regulated by dietary Zn intake. ZnT-2 may involve in Zn efflux or uptake into vesicles in intestine, kidney, and testis. ZnT-3 involves in Zn uptake into vesicles in neurons and possibly in testis. ZnT-4 is also an exporter and is highly expressed in mammary gland and brain (Cousin and McMahorn, 2000; McMahorn and Cousin, 1998; Liuzzi et al., 2001).

Distribution and Storage of Zinc

Zinc has been found in every tissue in the body. High concentrations of Zn have been found in the hair, wool, and skin. Bone tends to accumulate Zn more than liver (McDonald, 1995). The Zn content of most soft tissues including muscle, brain, lung, and heart are relatively stable. This soft-tissue Zn does not respond to or equilibrate with other Zn pools to release Zn when dietary Zn intake is low. Furthermore, while Zn is found in bones as a part of apatite, bones release the mineral very slowly and cannot be depended on as a supplier of Zn during

deprivation. Instead, when dietary Zn intake is insufficient to meet the body's needs, plasma Zn metalloenzymes and metallothionein provide Zn. Catabolism of selected "less essential" Zn metalloenzymes as well as liver metallothionein occurs so that Zn can be redistributed to meet particularly crucial needs for the mineral. Metallothionein is found in most tissues of the body, including the liver, pancreas, kidney, intestine, and red blood cell (Gropper et al., 2005).

Zinc Excretion

The major route of excretion is via feces, with smaller amounts eliminated in urine (McDowell, 2003). Endogenous Zn in the form of metalloenzymes is secreted by the salivary glands (canine is not found), intestinal mucosa, pancreas (main source), and liver into the GIT. Although some of this Zn is reabsorbed, some also is excreted in the feces. Zinc is also contributed to the GI lumen by sloughed intestinal cells (Gropper et al., 2005). Fecal Zn consists mostly of unabsorbed dietary Zn with a small amount of endogenous origin secreted into the small intestine (Underwood, 1977). Fecal Zn increases with increased Zn intake whereas urinary Zn does not very appreciably with dietary changes (McDowell, 2003).

Lowe et al. (1994b) found that dogs fed ZnAAC had lower ($P < 0.05$) fecal Zn excretion than dogs fed ZnO. Moreover, when Ca level in the diet was increased, dogs fed ZnO diet resulted in increased fecal Zn excretion but dogs fed ZnAAC diet was not affected from counteract antagonist. The amount of Zn eliminated via the feces and contamination of the environment were reduced when supplement with ZnMet as compared to $ZnSO_4$ (Rupic et al., 1997). Ahn et al. (1999) reported that pigs fed Zn methionine chelate had lower ($P < 0.01$) fecal Zn than pigs fed ZnO. Borges and Oliveira (2003) and França et al. (2005) reported that cats supplemented with Zn proteinate had greater ($P < 0.05$) Zn absorption and retention than $ZnSO_4$. Zinc retention in the body was not different ($P > 0.05$) when pigs fed Zn proteinate diet at lower Zn level than pigs fed ZnO diet (Calrson et al., 2004).

Physiological Functions of Zinc

Zinc has many seemingly divergent functions, probably due to the numerous metalloenzymes of which it is a component (Gropper et al., 2005). Therefore, the main physiological functions of Zn can be classified as follows:

1. Enzymes

Zinc provides structural integrity to the enzyme by binding directly to amino acid residues and/or participates in the reaction at the catalytic site. At least 70 and perhaps over 200 enzymes from every enzyme class have been shown to require Zn (Gropper et al., 2005). Zinc metalloenzymes present in all of the enzyme classes as shown in Table 1.

Zinc metalloenzymes, for example, alkaline phosphatase (ALP) plays a role in hydrolyzing monoesters of phosphates from various compounds. It contains four Zn ions per enzyme molecule. Two of the four ions are required for enzyme activity. The other two are needed for structural purposes (Gropper et al., 2005; O'Dell et al., 1979). Alkaline phosphatase is associated with bone metabolism. Three isozymes of ALP are known to exist in canine serum, including bone ALP (BALP), liver ALP, and corticosteroid-induced ALP (CALP). High levels of ALP are normal in growing puppies due to normal bone growth (Swanson et al., 2004). In addition, serum or plasma ALP has been the most commonly used enzyme and as good indicators for Zn status assessment (McDowell, 2003; Ziegler and Filer, 1996). Ahn et al. (1999) found that pigs fed Zn methionine chelate treatment had greater ($P < 0.01$) serum ALP activity than pigs fed ZnO. Serum or plasma ALP activity increased ($P < 0.01$) with increased Zn intake (Ahn et al., 1999; Revy et al., 2002).

2. Hormones

Zinc has many biologically significant interactions with hormones. It plays a role in the production, storage, and secretion of individual hormones (McDowell, 2003). Zinc serves as a necessary structural component of DNA-binding proteins (also called transcription factors) that contain Zn-fingers. Zinc-fingers is a term used to indicate the configuration of the proteins, which look like fingers, and the presence of the mineral Zn bound to the protein. DNA-binding proteins that contain

Zn fingers also bind other substances, such as retinoic acid, thyroxine or steroid hormones such as androgen, estrogen, and progesterone. Thus, hormones bind to specific protein-containing Zn-fingers. In the presence of Zn, which is required for the binding of the protein to the DNA, the protein (with the hormone attached to it) binds to the DNA to affect gene expression (Gropper et al., 2005). Zinc is a component of insulin (Pond et al., 1995). Kaji (2001) found that Zn functions in the release of growth hormone from the somatotrophs. Growth hormone might promote intestinal absorption of Zn and/or promote Zn uptake of hair root cells. Zinc has an important role in IGF-I synthesis can be impaired by Zn deficiency.

Table 1 **Zinc metalloenzymes in the six enzyme classes**

Class	Enzyme
Oxidoreductase	Alcohol dehydrogenase
	Superoxide dismutase
	Malic dehydrogenase
	Lactic dehydrogenase
Transferase	RNA polymerase
	DNA polymerase
	Reverse transcriptase
Hydrolase	Alkaline phosphatase
	Carboxypeptidase
	Collagenase
Lyase	Carbonic anhydrase
Isomerase	Phosphomannose isomerase
Ligase	tRNA synthetase

Reference: Kidd et al. (1996)

3. Reproduction

Zinc is associated with testicular growth and development (McDowell, 2003), and spermatogenesis (Underwood, 1977). Zinc deficiency produces testicular atrophy and failure of spermatogenesis (O'Dell et al., 1979). Zinc affected on progesterone (hormone that maintained pregnant status in female) (Gropper et al., 2005). Abnormal estrous cycles, infertility and resorption of implantation sites were observed in Zn deficient rats (O'Dell et al., 1979).

4. Immune Response

Zinc has been shown to be an essential element in the maintenance of cell-mediated immune functions. It is required enzymatically for the production of B-cells and T-cells. When there is a deficiency of Zn, B- and T-cell production is impaired with a resulting reduction in immunity (Albion, 1992). Zinc is an essential cofactor for the thymic hormone thymulin. Differentiation of immature T-cells in the thymus is induced by thymulin (Rink and Kirchner, 2000). Zinc is important for leukocyte functions (Rink and Gabriel, 2000; Rink and Kirchner, 2000). Zinc deficiency caused rapid atrophy of the thymus and interfered with T-cell helper function (O'Dell et al., 1979). The experimental studies with weanling pigs, Ahn et al. (1998) reported that pigs fed ZnMet treatments had higher ($P < 0.05$) serum IgG than ZnO treatments. High serum IgG indicates improvement in the immune function. In vitro study by van Heugten et al. (2003) found that when mitogen stimulated by pokeweed mitogen, lymphocyte proliferation was greater ($P < 0.05$) in pigs fed the ZnMet diet than pigs fed the $ZnSO_4$ diet.

5. Hair and Skin

Zinc plays a crucial role in skin nucleic acid and collagen synthesis (McDowell, 2003; O'Dell et al., 1979). On the other hand, Tomlinson et al. (2004) reported that Zn has a role in three key functions in the keratinization process as follow:

1) Catalytic roles

Catalytic roles are found in enzymes such as RNA nucleotide transferases, RNA polymerase, ALP, carboxypeptidase, alcohol dehydrogenase,

and the carbonic anhydrase. As indicated earlier, the presence of RNA, DNA, ascorbic acid, free aldehyde groups, and ALP in keratinizing cells serves as a positive indicator of intense cellular activity. These catalytic enzymes are Zn metalloenzymes and, such as, are dependent upon Zn as an activator, and thus an integral component in the differentiation of keratinocytes.

2) Structural roles

Zinc also plays a key role in the formation of the structural proteins during the keratinization process. Zinc-finger proteins are involved in functions requiring protein-to-protein interactions, most of which are thought to affect cellular differentiation or proliferation. Interestingly enough, Zn-finger proteins are thought to have the following general structure: $-C-X_2-C-X_n-C-X_2-C-$, where C designates Cys and X designates other amino acids. The favored pentapeptide sequence Cys-Gln-Pro-(Ser, Thr)-Cys was identified in the α-helix chain of hard mammalian keratins. They postulated that the Cys-favored positions may form the β-bend conformation, which is stabilized by a disulfide linkage between Cys residues. Therefore, it is postulated that insufficient Zn status may decrease the formation of Zn-finger proteins and thus the formation of keratin filaments needed in the developing keratinocyte.

3) Regulatory roles

Zinc plays a role in regulation of differentiating keratinocytes. It regulates calmodulin, protein kinase C, thyroid hormone binding, and inositol phosphate synthesis. Calmodulin is responsible for binding Ca^{2+} and carrying it into the cytosol of the cell when activated. This is important in the final step of the developing keratinocyte because Ca^{2+} activates epidermal transglutaminase. Protein kinase C (which also Ca^{2+} dependent) is responsible for phosphorylation of proteins, thus adding available energy to the differentiation process. Thyroid hormone acts to regulate the action of calmodulin and protein kinase C. Inositol phosphate acts to increase Ca^{2+} by mobilizing the ion from intracellular stores, primarily from the endoplasmic reticulum.

In more severe Zn deficiency, scaling and cracking of the paws with deep fissures develop, in addition to loss of hair and dermatitis (McDowell, 2003). In dogs, Zn supplementation improved hair coat, gloss, and decreased transepidermal water loss (Marsh et al., 2000). Dogs fed ZnAAC diet resulted in the Zn deposited in hair is about two times greater ($P < 0.05$) than dogs fed ZnO diet. The hair growth rate was greater ($P < 0.05$) in dogs fed diet containing ZnAAC than ZnO. With increasing dietary Ca, dogs fed diet supplemented ZnO resulted in decrease ($P < 0.05$) both Zn deposited in hair and hair growth rate when compared to ZnAAC (Lowe et al., 1994b). Lowe and Wiseman (1998) found that increasing ZnAAC level in diet had more Zn deposition in hair and hair growth than ZnO when compared at the same level of Zn supplementation. França et al. (2005) shown that cats fed diet supplemented with Zn proteinate had greater ($P < 0.05$) deposition of Zn in hair than ZnO and $ZnSO_4$. Kuhlman and Rompala (1998) reported dogs fed partial replacement of inorganic Zn, Mn, and Cu with proteinated forms of Zn, Mn, and Cu in diet resulting in hair appearing smoother and less fragmented than dogs fed only inorganic form of these minerals (Figure 6).

(a) control diet (b) chelated diet

Figure 6. Comparison of scanning electron microscopy photographs of a strand of hair from dog fed the control diet (containing only inorganic forms of Zn, Mn, and Cu) versus chelated diet (containing both inorganic and proteinated forms of Zn, Mn, and Cu) (Kuhlman and Rompala, 1998)

6. Growth

Physiological functions that are mentioned previously had associated with growth. Zinc plays a role in bone formation process and tissues growth. It associated with control of protein synthesis and anabolism and catabolism of nucleic acids (Gropper et al., 2005). Yu et al. (2005) reported that mice fed ZnMet diet had higher ($P < 0.05$) level of IGF-I mRNA, weight gain, and food intake than mice fed $ZnSO_4$ diet. Young pigs fed ZnMet diet had greater ($P < 0.05$) feed efficiency (FE) than pigs fed ZnO or $ZnSO_4$ diet (Hahn and Baker, 1993). Growth performance (Ward et al., 1996) and FE (Carlson et al., 2004) were not differ ($P > 0.05$) when starter pigs fed diet containing ZnMet at lower level than ZnO.

Zinc Requirements of Dog

Zinc requirements are various with age, physiological state, environmental factors, and health of the animal (McDowell, 2003). Young growing animals and reproducing adults require higher levels of Zn than do nonreproducing adults. Zinc requirements may also be significantly influenced by various dietary ingredients that was mentioned previously as they can decrease the absorption and utilization of Zn (Colombini, 1999).

Now, the dog has evolved to become one of the most variable animal species. Anywhere from 300 - 400 distinct dog breeds exist, which vary dramatically in size and which display an astounding amount of variation in coat type, coat colour, and general morphology (Zgurski, n.d.). Classification of dog breeds by body size are small, medium, large, and giant breed. Whether classification by coat are three types. Description the following types of coat in dogs: 1) short hair such as Rottweiler and Fox terriers; 2) normal hair such as German Sheep Dog and Alsatian; and 3) long hair such as Pomeranian and Poodle (Rook and Walton, 1965). At present, informations about Zn requirements of each dog breed are not found but it is believed that each breed has different Zn requirement. The information of Zn relating to hair growth was mentioned previously. Consequently, long-haired breeds is believed to have greater Zn requirements than short- and normal-haired breeds.

In addition, National Research Council (NRC) has established Zn requirement guidelines for the canine diet. These guidelines, however, are primarily based on data extrapolated from other species and may not be precise (Colombini, 1999). NRC (2006) recommended Zn requirement for adult dogs food formulation was 60 ppm DM for diet had a metabolizable energy (ME) 4.0 kcal/g DM. Increasing dietary Zn above the allowance recommended, however, remains common practice to counteract antagonist effects on Zn absorption in many commercial diets (Lowe et al., 1994b). The AAFCO's nutrient profiles provide recommendation for practical minimum and maximum levels of nutrients in commercial pet foods. All pet food companies required to use this profile rather than the NRC's recommendations when formulating dog foods to met established nutrient levels (Case et al., 2000). The AAFCO (2000) recommended minimum Zn requirement for maintenance was 120 ppm DM. While maximum Zn requirement was 1000 ppm DM. Both minimum and maximum Zn requirements were extrapolated from diet had a ME 3.5 kcal/g DM.

Zinc Deficiency in Dog

Hair coat and skin changes are usually the first clinical signs of Zn deficiency. These signs have been described as alopecia, dull, coarse hair coat and skin lesions that show parakeratosis and hyperkeratinization. Other clinical signs include growth retardation, gastrointertinal disturbances (e.g., diarrhea), and impaired reproductive performance (e.g., reduce the size of litters) (Case et al., 2000). Colombini (1999) reported that Zn-responsive dermatosis is typically classified into two distinct clinical syndromes. Syndrome 1 includes focal erythemia encircle the eyes, ears, nose, mouth and pressure points, alopecia, dull and dry hair coat. These syndromes occur primarily in Sibirian Huskies and Alaskan Malamutes, although other breeds may be affected. They have a genetic defect that results in diminished intestinal Zn absorption. Cutaneous lesions frequently occur during puberty. Syndrome 2 includes anorexia, stunted growth, and dermatological lesions often resembly those seen in Syndrome 1 but occur in rapidly growing puppies fed

Zn-deficient diet or diets containing substances which inhibits the absorption and utilization of Zn. However, these syndromes can be treated by Zn supplementation.

Zinc Toxicosis in Dog

Animals exhibit consiferable tolerance to high intakes of Zn, the extent of the tolerance depending partly on the species but mainly on the nature of the diet, especially its relative contents of Ca, Cu, Fe, and Cd, which Zn interacts in the processes of absorption and utilization (McDowell, 2003). Most documented canine Zn toxicoses are associated with the ingestion of pennies. The pennies contain an alloy consisting of approximately 98.4% Zn. Other causes of Zn toxicoses in dogs include cage wires and cage nuts, ZnO ointment, and brass buttons. Symptom exhibition such as anorexia, vomiting, depression, fever (39.9°C), icterus, and intravascular hemolysis (Hammond et al., 2004; Volmer et al., 2004). Therefore, no report causes of Zn toxicosis in dogs from food.

Hair

1. The Importance of Hair

The skin of the dogs is completely covered with hairs except for the nose, foodpads, and mucocutaneous junctions (Muller and Kirk, 1976). Hair is important in thermal insulation, sensory perception, and as a barrier against chemical, microbial, and physical injury to the skin. In pet animals, hair is of great aesthetic importance to owners, and a source of great concern when it is not normal. The ability of the coat to regulate body temperature correlates closely with length, thickness, density per unit area, and medullation of individual hair fibres. In addition, coat colour is of some importance in thermal regulation (Tscharner and Halliwell, 1990).

2. Hair Growth and Cycle

Hairs do not grow continuously, but in cycles (Muller, 1995; Tscharner and Halliwell, 1990). Hair is produced by division of cells in the base of the stocking-like inpushing of the epidermis known as a hair follicle. The wall of the follicle, the outer root sheath, is continuous with the superficial epidermis and at the base of the

follicle fits over a small knob known as the dermal papilla. The follicle has a cycle of activity. During the active period or *anagen* hair is produced by division of cells in the matrix surrounding the dermal papilla. At the end of the active phase the middle region of the hair bulb starts to become constricted and above this constriction the base of the hair becomes expanded and keratinized to form a 'club'. At this transitional stage or *catagen*, the connective tissue sheath of the follicle, in particular the vitreous membrane, thickens enormously and becomes released from its epidermal investment and subsequently the hair migrates towards the surface and the epithelial strand shortens to form a little nipple called the secondary germ; this resting stage is called *telogen*. When the next period of activity starts, the secondary germ elongates by cell division, grows downwards to enclose the papilla, and gives rise to a new hair bulb. The newly formed hair subsequently emerges alongside the old club, which is then lost (Rook and Walton, 1965). The hair cycle is shown in Figure 7.

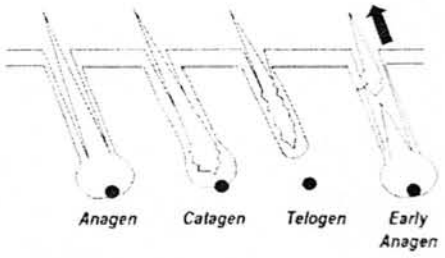


Figure 7. The hair cycle. All hairs go through a cycle of active growth (anagen), shrinkage (catagen), and transient inactivity (telogen). At the beginning of the new anagen phase, the old telogen hair falls out (arrow) (Melman, 1994)

In normal dogs, this cycling proceeds like clockwork. Every time a hair is lost, a new one soon replaces it. The relative duration of the phases of the cycle varies with the age of the individual, region of the body, breed, sex, and can be modified by a variety of factors, both physiological and pathological. The hair cycle and thus, the hair coat, are controlled by photoperiod, ambient temperature, nutrition, other environmental factors, hormones, general stage of health, genetics,

and poorly understood 'intrinsic factors' (Melman, 1994; Tscharner and Halliwell, 1990).

3. Hair Division

Rook and Walton (1965) reported that a hair may be divided into three parts; 1) hair shafts, they emerge through a pore in the skin (Melman, 1994); 2) hair follicle, it has very large blood-supply that provide nutrient; and 3) hair papilla (Figure 8). Dogs have compound hair follicles, meaning multiple hair shafts (sometimes as many as 20) exit through each pore. There is marked variability in the length and diameter of the hair shafts in dogs (Lowell, 1993; Melman, 1994).

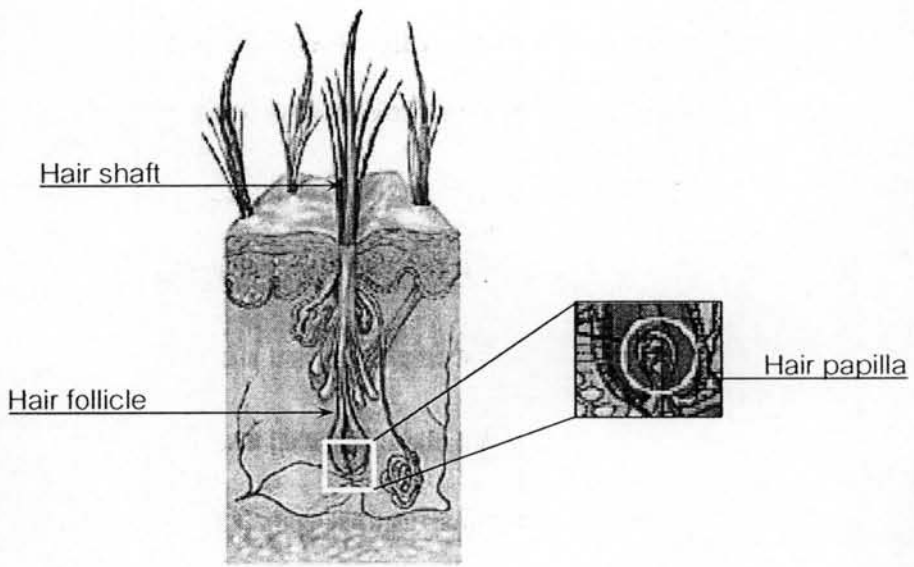


Figure 8. The division of dog's hair (modified from O'Keefe,n.d.)

4. Biochemistry Properties of Hair

Between 90 and 99% of the dry weight of hair is keratin, hard α-keratin. Species hairs can show major differences in the type and amount of high-sulfur proteins. The most important requirement for follicular protein synthesis is the sulphhydryl-containing amino acid *cysteine*, as it ultimately forms the stable disulfide bonds of hair and it would affect to the rate of synthesis of high-sulfur hair matrix proteins (Tscharner and Halliwell, 1990).

As mention previously, forms and physiological functions of Zn involve with hair. Chelated form of Zn can be more absorbed and utilized than inorganic Zn. However, dog foods formulation cannot yet indicate appropriate level for Zn supplementation. Therefore, this study will be expected to find out the appropriate level of Zn supplementation in commercial dog foods that give great hair coat characteristics and Zn concentration in plasma and hair, and less fecal Zn excretion of dogs.