AVAILABILITY OF ZINC FROM AN AMINO ACID CHELATE IN ZN DEPLETED PIGS

by

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(Abstract)

This study was conducted to compare the availability of Zn from two Zn sources, an amino acid chelate and $ZnSO_4$. In three experiments, 78 Zn depleted and 24 Zn adequate pigs were used. Pigs were depleted of Zn by feeding an isolated soy protein, semipurified diet containing 17 ppm Zn. Of the 78 depleted pigs, 60 pigs were Zn repleted. During Zn repletion in Exp. 1, depleted pigs were fed the low Zn diet supplemented with 5, 15, or 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC). In Exp. 2 and 3, low Zn diets were only supplemented with 45 ppm Zn. Zinc adequate pigs, used in Exp. 1 and 2, were fed the 45 ppm supplemental Zn diets. To evaluate differences in site and rate of apparent Zn absorption, chromic oxide was added to the diets of depleted pigs in Exp. 1 and 3. In all experiments, a 24-d period was sufficient to severely deplete the porcine body Zn stores, and to cause parakeratosis and growth retardation. Serum Zn concentrations and serum ALP-activities of depleted pigs dramatically decreased ($P < .01$) during the first 14 d of Zn depletion. At the end of Zn depletion, Zn contents in liver, kidney, pancreas, brain, and small intestine tissues of pigs fed the

low Zn diet were reduced ($P < .01$) by 10 to 40 % compared with the adequate pigs fed the $ZnSO_4$ and ZnAAC diets. In Exp. 2, the growth retardation was associated with a low ($P < .05$) serum mitogenic activity and pituitary RNA content of depleted pigs compared with pair-fed adequate pigs. Moreover, the growth hormone mRNA fraction tended to be reduced ($P < .10$) for the Zn depleted pigs. In Exp. 1, the apparent absorption of Zn was higher ($P < .01$) for pigs fed ZnAAC compared with the $ZnSO_4$ group; however, this was not confirmed in Exp. 3 unless coefficients were corrected for Cr recovery. Furthermore, absorption of Zn occurred primarily within jejunal and distal segments of the small intestine. In the balance of Exp. 3, disappearance rates of Zn, Cu, Fe and DM were higher ($P < .01$) in depleted pigs fed ZnAAC compared with $ZnSO_4$. The recovery of Cr also was different ($P < .01$) between pigs fed the $ZnSO_4$ (87 %) and ZnAAC (70 %) diets. Moreover, the moisture content of the fecal matter was 11 % higher ($P < .01$) for the ZnAAC group compared with pigs fed $ZnSO_4$. In Exp. 1, depleted pigs fed the 15 ppm $ZnSO_4$ and ZnAAC diets regained their ability to grow, however, replenishment of body fluid and tissue Zn pools did not occur within the 24-d Zn repletion period. Both the 5 ppm $ZnSO_4$ and ZnAAC groups did not respond to Zn repletion within a 12-d period. In all experiments, the rate and degree of repletion of body fluid and tissue Zn stores was not different between pigs fed the 45 ppm $ZnSO_4$ and ZnAAC diets, although a higher ($P < .05$) serum mitogenic activity was observed for the adequate pigs fed ZnAAC compared with $ZnSO_4$. In conclusion, an amino acid chelate did not improve growth, or rate and degree of replenishment of body fluid and tissue levels of Zn compared with pigs fed $ZnSO_4$. However, ZnAAC may have influenced intestinal luminal conditions since a higher rate of disappearance of Zn, Cu, Fe, Cr, and DM was measured.

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It is not customary in Holland to acknowledge contributions of parents and other loved ones at times of a professional achievement. For this occasion though, I would like to write down the words of goodbye spoken by my father at the beginning of my journey: "toi, toi, toi". Mom and dad, these words kept me going!

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CHAPTER I

Introduction

In the 1990's, the swine industry in the United States finds itself at the brink of dramatic change. Confinement rearing of pigs will increase in popularity, thereby intensifying the use of computers as a management tool. Consumer demand for lean pork will force the packing industry to completely switch from payments on a live weight basis to carcass merit. Environmental concerns are rising and will pressure the swine industry to employ production methods which generate less waste. Furthermore, the still infantile animal well-being issue is likely to mature and needs to be addressed by the scientific community.

The anticipated changes in the swine industry represent a challenge. For the swine nutritionist in particular, the transition will introduce new avenues of research of which many should focus on efficient utilization of nutrients.

This study is part of a long term research project in which methods for enhancing the utilization of macro and micro minerals will be examined. Even though minerals are only supplemented in small amounts, they are likely to become an environmental concern. In addition, identification or development of highly available sources of minerals may effectively reduce the cost of swine production.

Recently, inclusion of organic Zn sources in swine diets has been propagated by certain segments of the feed industry. Organic forms of Zn are believed to be better absorbed, and therefore supply more available Zn than the commonly used inorganic Zn sources.

To test this hypothesis, we compared the availability of Zn from an organic amino acid chelate and zinc sulfate using Zn depleted pigs.

The specific objectives in this research were:

(1) to evaluate the sensitivity of conventional and novel indicators for assessing Zn status during experimental Zn depletion of pigs (Chapter III);

(2) to compare the rate and gastrointestinal site(s) of Zn absorption of a Zn amino acid chelate and $ZnSO_4$ during repletion of Zn depleted pigs (Chapter IV);

(3) to compare the effectiveness of a Zn amino acid chelate and $ZnSO_4$ in repleting body fluid and tissue Zn stores, and in restoring total serum growth factor activity of Zn depleted pigs (Chapter V).

CHAPTER II

Review of Literature

Introduction

Growth retardation and an abnormal hair coat, induced in rats by feeding a low Zn purified diet (1.6 ppm Zn), were the first clinical signs associated with dietary Zn deficiency (Todd et al., 1934). Two decades later, a direct relationship between Zn and growth was reported for swine (Tucker and Salmon, 1955). In addition to impaired growth, Tucker and Salmon (1955) also observed a dermatosis in the Zn deficient pigs, coined "parakeratosis" by Kernkamp and Ferrin in 1953. Parakeratosis is the classic characteristic associated with severe Zn deficiency in swine (NRC, 1988).

Since the recognition of Zn as an essential nutrient, many researchers have studied the role of Zn in biology and nutrition. In the first section of this review the biology of Zn is examined. This is followed by an overview of our current understanding of absorption and bioavailability of Zn. Lastly, the possible biological value of dietary complexes and chelates of Zn is discussed.

Biology of Zinc

Zinc in Porcine Tissues and Fluids. The concentration of Zn in the whole body of the pig, expressed on an fat-free basis, is 25 mg/kg (Spray and Widdowson, 1950). This is within the range of 20 to 30 mg/kg reported for fat-free bodies of

rats, cats, human (Spray and Widdowson, 1950), sheep (Grace, 1983) and dairy cows (Miller, 1974). Concentrations of Zn in tissues, fluids, bone and integuments of the pig are presented in Table 1. Similar distributions of Zn have been reported for rats, sheep, cows, monkeys and human (Hambidge et al., 1986; Jackson, 1989).

In pigs, the highest concentration of Zn is found in hair. However, proportionate to the Zn content of the whole body the total amount of Zn in hair is small. The largest pool of Zn, approximately 60%, is found in skeletal muscle tissue because of its bulk and fairly high Zn concentration (Jackson, 1989). The concentration of Zn varies with the type of skeletal muscle. The highest amounts of Zn are found in red skeletal muscle and the lowest in white skeletal muscle (Cassens et al., 1967). The remainder of the Zn pool of the body is primarily located in bone and organs. A small proportion of total body Zn is found in body fluids which contain very low concentrations of Zn (Hambidge et al., 1986).

The total Zn content of the body on a weight basis remains fairly constant from birth to maturity (Spray and Widdowson, 1950). The proportion of Zn in the body found in the liver gradually increases during suckling. After weaning, the Zn content rapidly decreases to plateau at approximately the level of Zn present in the liver of pigs at birth (Spray and Widdowson, 1950).

Intracellular Distribution of Zn. As shown in Table 1, Zn is mostly present in tissues and bone, which suggests that Zn is located primarily within the cell. In murine liver, the largest proportion of intracellular Zn was found in the light fraction of organelles other than mitochondria and nuclei (Bartholomew et al., 1959). In porcine muscles, the largest amount of Zn was found in the heavy fraction of myofibrils and nuclei (Cassens et al., 1967). The level of Zn in the heavy fraction of red skeletal muscle was almost four times as high as in white skeletal muscle.

Table 1. Concentration of Zn in body fluids and tissues of fast growing pigs[a]

Item	Concentration
Blood:	
Plasma (μg/dL)	74
Serum (μg/dL)	60
Erythrocytes (μg/g packed cells)	7.7
Leucocytes[b]	21.5
Tissues[b]:	
Bone	113
Brain	70
Heart	96
Kidney	141
Liver	151
Red muscle	137
Mixed muscle	89
White muscle	67
Pancreas	161
Spleen	107
Integuments[b]:	
Hair	201
Skin	28

[a]Data compiled from Cassens et al. (1967), Crofton et al. (1983), Hoekstra et al. (1956, 1967), Miller et al. (1968) and Zhou et al. (in preparation)
[b]Data of leucocytes, tissues and integuments are expressed as ppm on a DM basis

Concentrations of Zn in the light fraction of mitochondria, microsomes and supernatant were equally low for both muscle types. This suggests that Zn in red skeletal muscle is primarily associated with myofibrils (Cassens et al., 1967). These studies indicate that intracellular Zn is ubiquitously distributed within the cell. The proportion of Zn located at the different intracellular sites may not only vary among tissues, but also between cells of similar tissues.

The nature of intracellular Zn is much harder to determine. Most biochemical techniques currently available involve destruction of the cell. This permits Zn to exchange ligands prior to analysis of Zn binding compounds (Jackson, 1989). Nevertheless, it is apparent that Zn is part of many cellular metalloenzymes (Galdes and Vallee, 1983) and binds readily to the thiolate ligands present in the cellular protein metallothionein (Vasak and Kagi, 1983).

The distribution and nature of intracellular Zn is shown in Figure 1. Intracellular Zn appears to exist largely bound to cell proteins (Williams, 1984). This does not imply that existence of substantial amounts of free Zn or Zn bound to amino acids can be ruled out (Jackson, 1989).

Biological Functions of Intracellular Zn. To date, Zn has been proposed to play a role in the following biological processes: (1) catalysis, (2) structural arrangement of protein, and (3) regulation of cellular events (Williams, 1989). For each of the processes, Zn exerts its biological activity almost entirely as part of complex molecules.

The catalytic function of Zn is clearly demonstrated in the enzyme carbonic anhydrase (Galdes and Vallee, 1983). The only physiological reaction known to be catalyzed by carbonic anhydrase is the reversible hydration of carbon dioxide (H_2O

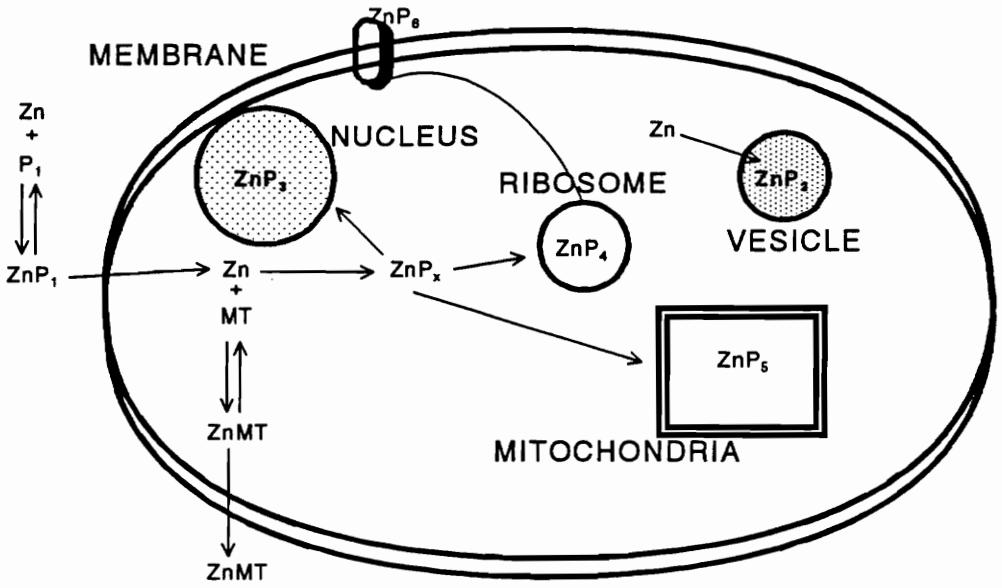

Figure 1. Outline of the distribution of intracellular Zn (Williams, 1984). ZnP_1, extracellular protein carrier. MT, metallothionein. Vesicle containing ZnP_2 possibly exports enzymes or hormones. Nuclear, ZnP_3, and ribosomal, ZnP_4, proteins may be involved in polymerization, catalysis or protection. ZnP_6 is, f. e., alkaline phosphatase

+ $CO_2 = H^+ HCO_3^-$). The Zn ion is thought to participate in the first step of the catalytic reaction. Basically, Zn functions as an electron acceptor, or Lewis acid, and binds to the H_2O molecule. Due to the neutral imidazole ligands of the enzyme, the complex $Zn(H_2O)$ attains maximum acidity making ionization of H_2O to OH^- possible at pH 7 (Williams, 1989). The reactivity of the nucleophilic OH^- group is sufficient for carbonic anhydrase to attack the electrophilic CO_2 molecule. As a result, the end product HCO_3^- is formed (Galdes and Vallee, 1983). Zinc is thought to behave in a similar way in other Zn metalloenzymes which harbor Zn at the active site.

Zinc may play a structural role in enzymes whenever it is located in a site not critical for catalysis. Furthermore, Zn is considered of structural importance to other protein such as insulin and growth hormone. In a sense, Zn can be viewed as a replacement for a disulphide bond. The disulphide bridge is a common feature in many proteins providing stability by interlinking polypeptide chains (Stryer, 1988).

A disulphide bridge may not be the best option for the protein. The sulfur containing amino acids of the protein chain may be protonated in a reducing environment after which the disulphide bridge is lost. Furthermore, the disulphide bridge is very rigid allowing little motion about itself thereby restricting protein confirmation. Conversely, Zn is insensitive to reducing environments and has very little stereochemical demand (Williams, 1984). Thus, Zn appears to provide enzymes and other Zn containing proteins with stability of their conformational modes at various pH's without causing much steric hindrance.

A possible cooperative role of Zn and enzymes in regulation of metabolic processes and synthesis has been recognized (Jackson, 1989). Furthermore, evidence has been presented that Zn is involved in gene expression of

metallothionein (Seguin and Hamer, 1987). Recently, Cousins and Lee-Ambrose (1992) used rats to investigate the interactions of dietary Zn intake, nuclear Zn uptake and metallothionein gene expression. They reported that increases in dietary Zn were proportional to nuclear uptake of ingested Zn as well as to metallothionein gene expression in the kidney, liver, intestine, spleen and heart. Several Zn binding protein fractions were isolated using Heparin-Sepharose chromatography and southwestern blotting. One of these fractions was able to bind an oligonucleotide in addition to Zn. The oligonucleotide was a DNA-fragment of a transcription factor of the metallothionein gene (Cousins and Lee-Ambrose, 1992).

It is not always easy to distinguish between the catalytic, structural and regulatory functions of Zn. A good illustration is provided by Zn present in RNA and DNA polymerases. First, Zn may act catalytically by binding substrate, primer or template. Secondly, Zn may act in a regulatory manner by supplying specificity to proteins involved in gene replication and transcription. Finally, Zn may act structurally by maintaining polymerase confirmation (Wu and Wu, 1983).

Intracellular Mineral Interactions. The functions exhibited by Zn can be extended to Mn and Fe. Other trace minerals, Co, Ni, Cu and Mn are believed to be strictly involved in catalytic processes (Williams, 1984). Of all minerals, Cu is chemically most favored to bind with metal ligands present in specific sites of protein. Thus, Zn can compete for protein binding with Cu at high free Zn to free Cu ratios. The order of diminishing thermodynamic preference of minerals is Cu, Zn, Ni, Co, Fe, Mn, Mg and Ca (Williams, 1984). Selective uptake, secretion and intracellular binding are a few examples of methods the cell can employ to obtain a high concentration or the preferred metal at a particular cellular site.

Zinc Homeostasis

Homeostasis can be considered effective when the animal is able to maintain optimum health and function (Aggett, 1991). Initially, the animal is able to maintain Zn homeostasis by varying the rates of Zn absorption and excretion (Figure 2). In the short term the animal can further adjust by redistribution of Zn to the vital pools or by sequestering Zn. Conditions of long term deprivation or excess of dietary Zn lead to inadequacy of processes involved in maintaining Zn homeostasis (Figure 2).

The mechanisms which enable the animal to maintain Zn homeostasis are not exactly understood (Aggett, 1991). This lack of understanding is an important factor contributing to the problems encountered in determining Zn status.

Assessment of Zn Status. Levels of plasma and serum Zn, and activities of Zn containing metalloenzymes are frequently obtained in studies with humans (Prasad et al., 1971). When animals are used, these easily obtainable indicators of Zn status are often supplemented with measurements of Zn content and Zn metalloenzyme activities in various tissues (Giugliano an Millward, 1984). It is questionable, though, whether these indicators are sensitive enough to provide the accuracy required for a reliable interpretation of Zn status.

Blood contains only a small proportion of the whole body Zn content (Jackson, 1989). Consequently, Zn levels of plasma and serum do not only respond rapidly to increases in dietary Zn intake, but also to minor catabolic processes occurring in tissues with large Zn stores.

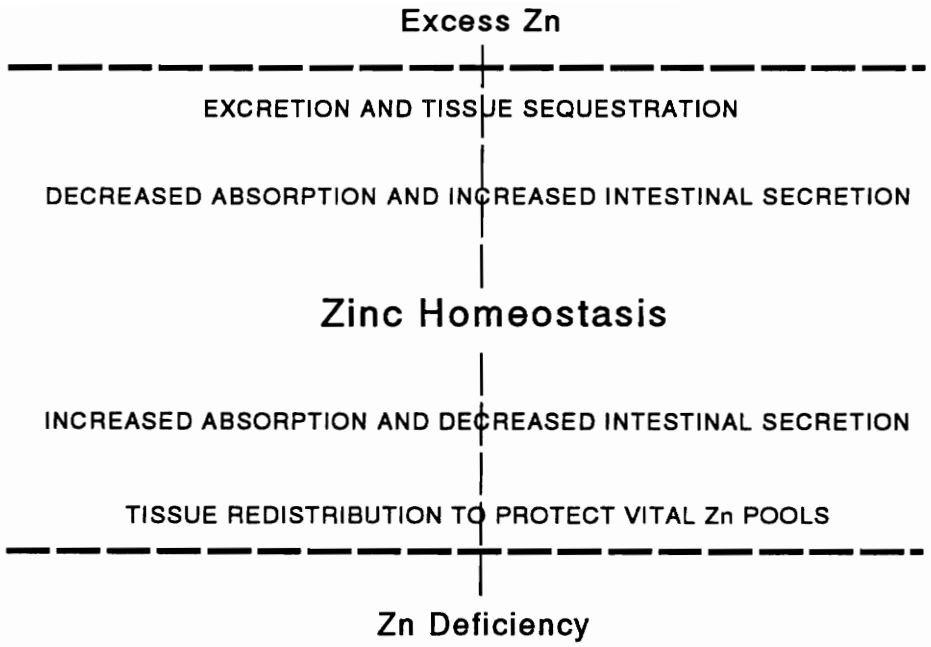

Figure 2. Adaptive processes used to maintain zinc homeostasis during short and prolonged periods of low or high dietary Zn intake

The effect of dietary Zn intake on tissues is substantial, but not uniform among tissues. Lower levels of Zn have been observed in the pancreas, liver, kidney, heart, intestine, skin, hair and bones of Zn-deficient pigs compared with pair-fed control counterparts (Hoekstra et al., 1956, 1967; Miller et al., 1969; Crofton et al., 1983; Dørup and Clausen, 1991). No difference in Zn content was observed in skeletal muscle tissue (Crofton et al., 1983; Dørup and Clausen, 1991). The large Zn pool found in skeletal muscle appears to be important for the biological functioning of the animal.

The relationship between dietary Zn intake and tissue Zn levels was studied by Cousins and Lee-Ambrose (1992). Following an overnight fast, diets containing different levels of Zn were administered via a stomach tube to Zn adequate rats. Two hours after feeding, the largest portion of ^{65}Zn was found in the small intestine, followed by liver, bone marrow, bone, skin, kidney, serum, thymus and skeletal muscle (Cousins and Lee-Ambrose, 1992).

It may be argued that metabolically or functionally important Zn pools are located in those tissues and body fluids that are the least affected by dietary Zn intake. Sensitive Zn pools may be located primarily in tissues which strongly respond to dietary Zn intake. Many tissues and body fluids increase or decrease their Zn contents with variations of Zn intake. This suggests that the sensitive Zn pools are plenty but small.

In clinical nutrition, two approaches have been proposed to identify the early onset of Zn deficiency. The first method involves the extremely difficult task of locating the sensitive Zn pools. Thus far, attempts to identify these Zn pools have been fruitless.

An alternative approach is to diagnose factors involved in Zn redistribution. Some progress has been made in identifying a key factor involved in Zn redistribution, namely metallothionein (Golden, 1989). Metallothionein is a low molecular weight protein with a high metal binding capacity. Many tissues contain metallothionein, with the highest concentrations found in the liver, kidney, pancreas and small intestine (Cousins, 1985).

Recently, King (1990) proposed the use of a combination of plasma levels of Zn and metallothionein to accurately assess Zn status in men. Low plasma Zn and metallothionein levels would imply depletion of the exchangeable Zn pool as a result of inadequate Zn intake. Conversely, low plasma Zn in combination with high plasma metallothionein levels could be interpreted as occurrence of tissue redistribution of Zn. The latter condition is not necessarily caused by low Zn intake (King, 1990).

The discussion of how to effectively assess Zn status is most prevalent in clinical nutrition. A balanced diet is not available to every human and most of the time there is a lack of information on dietary intake. Consequently, cases of Zn deficiency are frequently reported (Prasad, 1988), especially if Zn demands are increased due to growth or pregnancy (Yasodhara et al., 1991).

The development of diagnostic tools which allow easy and accurate diagnosis of Zn status is essential. Without these indicators, it remains impossible to diagnose premature cases of Zn deficiency.

In animal nutrition, optimum levels of Zn have been defined for the different domestic species. Dietary recommendations of Zn are provided (NRC, 1979) and diets are formulated accordingly. Therefore, Zn deficiency is rarely observed in the modern livestock production and assessment of Zn status is not common practice.

Biological Adaptations during Zn Deficiency. Experimentally depleted laboratory animals have been used to enhance our knowledge of the biological effects of Zn deficiency. Early studies showed that Zn depletion reduces levels of Zn and Zn metalloenzymes in many tissues and body fluids (Hoekstra et al., 1956, 1967; Prasad et al., 1971). More recently, red blood cell membranes and immune response of severely dietary Zn deficient animals have been compared with assumed standards. The Zn deficient animals showed an increase in osmotic fragility of red blood cell membranes (Johanning et al., 1990) and a depression in humoral and cellular immune response (Gupta et al., 1985; Spears et al., 1991; Verma et al., 1988).

Of particular interest to many animal scientists are the studies which have investigated the relationship between dietary Zn intake and growth. Growth retardation observed at low intakes of Zn can only partly be accounted for by overall depression or feed intake. This was demonstrated by Miller et al. (1969). In their study, performance was determined for three groups of pigs receiving different amounts of dietary Zn. Pigs fed a Zn deficient diet had reduced gains and were less efficient than both pair-fed controls and control pigs with ad libitum access to feed. Feed efficiency was not different between the two control groups (Miller et al., 1969).

Growth retardation of Zn deficient animals has been associated with reductions in levels of blood insulin-like growth factor (IGF)-I (Cossack, 1986; Dørup et al., 1991) and insulin (Giugliano and Millward, 1987; Dørup et al., 1991). Serum growth hormone levels, however, were not different between the Zn deficient and pair-fed control rats (Dørup et al., 1991).

In fast growing animals, Zn deficiency primarily affects protein metabolism. Reduced protein accretion has been found to occur in skeletal muscle, heart, thymus (Giugliano and Millward, 1987; Dørup and Clausen, 1991), and small intestine tissues (Southon et al., 1986) of Zn deficient animals compared with Zn adequate controls. The reduction in protein growth of the small intestine was not associated with the viability of intestinal bacteria (Southon et al., 1986). A direct metabolic association between Zn deficiency and reduction in protein growth of tissues including the small intestine may be assumed.

The severity of the biological effect of Zn deficiency is indicative of the importance of Zn as a nutrient. The changes in growth, gross anatomy and histology are directly related to inadequate supply of intracellular Zn. Consequently, levels of Zn-metalloenzymes, Zn-proteins, and Zn-transcription factors are reduced thereby impairing the biological functioning of the animal.

Absorption of Zinc

The term "absorption" is generally used to describe an overview of nutrient balance. More precisely, the term "apparent absorption" is used when endogenous losses by fecal secretions are not accounted for. True absorption corrects the nutrient balance for endogenous losses occurring by intestinal secretions and mucosal sloughing (O'Dell, 1984).

The process of Zn absorption can be physiologically divided into two separate events. Firstly, uptake of Zn from the lumen into the cell, and secondly Zn transport from the cell into the circulatory system. Recently, Cousins (1989) has

reviewed the current knowledge on mechanisms suspected to be involved in Zn uptake and transport (Figure 3).

Uptake or cellular entry of Zn appears to occur by means of active transport and facilitated diffusion which are both saturable (Davies, 1980; Menard and Cousins, 1983; Blakeborough and Salter, 1987). A small portion of Zn uptake and transport is non-saturable occurring through simple diffusion (Steel and Cousins, 1985) and paracellular movement (Bronner, 1987).

The saturable uptake of Zn may involve low molecular weight ligands which complex Zn within the intestinal lumen. The Zn-ligand complex either enters the cell intact or donates Zn to a membrane bound receptor. The receptor releases Zn intracellularly.

Evidence presented thus far suggests that Zn absorption is initiated largely by saturable uptake of Zn from the intestinal lumen into the cell. Transport of Zn out of the cell into the vascular system was studied by Oestreicher and Cousins (1989) using basolateral membrane vesicles of rat intestine. Vesicular uptake of Zn was saturable and not affected by dietary Zn intake (Oestreicher and Cousins, 1989). This indicates that Zn may use a carrier-mediated mechanism to enter the blood circulation. The lack of effect of dietary Zn intake on vesicular Zn uptake further suggests that Zn absorption is not regulated at the basolateral membrane.

A regulatory role of intestinal metallothionein in Zn absorption was first proposed by Richards and Cousins (1975). Metallothionein appears to be synthesized in proportion to dietary Zn intake. After synthesis, intestinal metallothionein reduces Zn absorption by sequestering Zn within the enterocyte due to its higher affinity for Zn compared with other identified intestinal proteins (Starcher et al., 1980; Menard et al., 1981).

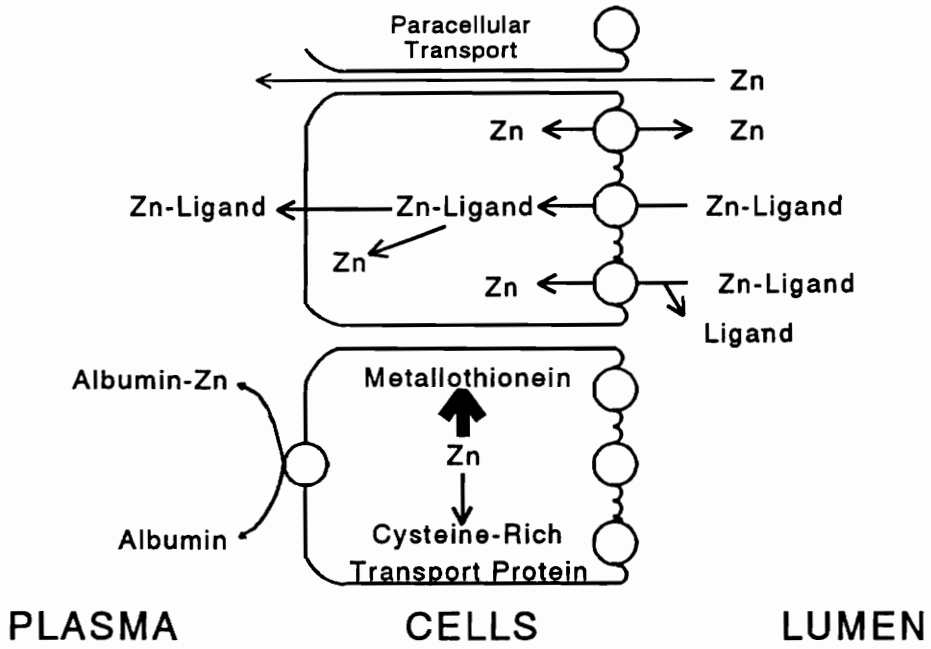

Figure 3. A model for zinc absorption (Cousins, 1989). Zinc uptake (top) may involve carrier-mediated and non-mediated mechanisms. Metallothionein is proposed to regulate Zn absorption (bottom) by competing for Zn with a cysteine-rich transcellular transport protein (Cousins and Lee-Ambrose, 1992)

Recently, Hempe and Cousins (1992) further developed their Zn absorption model describing the regulatory role of metallothionein (Figure 3). In their model, they suggest an interaction of a cysteine-rich intestinal protein, identified by Hempe and Cousins in 1989, with metallothionein. The intestinal protein is proposed to transport Zn transcellularly, from the intestinal brush border to the basolateral membrane. Binding of Zn to the transport protein, and thus absorption of Zn, is competitively inhibited by metallothionein (Hempe and Cousins, 1992).

The regulatory role of metallothionein has been partly challenged. Since Flanagan et al. (1983) only observed a transitory effect of dietary Zn level on metallothionein synthesis, they concluded that metallothionein synthesis was most likely induced by the nutritional stress associated with Zn deficiency. Dietary Zn intake did induce metallothionein synthesis in rats used by Coppen and Davies (1987), but only between Zn levels of 5 to 80 mg per kg diet. At higher levels of Zn, no further induction of metallothionein was observed.

After being absorbed, Zn most likely binds to the blood protein albumin (Smith et al., 1978). Albumin carries Zn to tissues which require Zn for metabolic processes. Excess Zn is secreted into the intestinal lumen, excreted by urine via the kidney or sequestered in bone and other tissues.

Site(s) of Zn Absorption. Many researchers have investigated the capacity of Zn absorption in various sites of the gastrointestinal (GI) tract. Some have reported that net Zn absorption in rats occurs primarily in the small intestine (Underwood, 1977) with negligible Zn absorption occurring in other segments of the GI-tract (Davies, 1980; Underwood, 1977). Others, however, did observe substantial Zn absorption in the large intestine of rats (Wapnir et al., 1985), pigs (Partridge, 1978), sheep (Grace, 1985) and cattle (Bertoni et al., 1976). Moreover, Zn absorption also

occurred anterior to the small intestine in chickens (Miller and Jensen, 1966) and dairy cattle (Miller and Cragle, 1965).

Duodenal and ileal segments of the small intestine have been suggested to function as primary sites of Zn absorption. Infusion of ^{65}Zn into the duodenum led to the highest ^{65}Zn recovery in blood, liver, kidneys and heart (Van Campen and Mitchell, 1965) or the whole body (Davies, 1980). With the use of an in vivo intestinal perfusion technique, the ileum was found to have the highest capacity for Zn absorption (Antonson et al., 1979). Conversely, Seal and Mathers (1989) reported that the rates of Zn transfer from everted gut sacs of duodenal, ileal and colonic segments of rats were not different. In their studies, analysis of intestinal tissues showed that Zn accumulation in everted duodenal sacs was twice as high as in either ileal or colonic sacs. Including dietary cellulose increased Zn uptake in everted colonic sacs of rats (Seal and Mathers, 1989). Although the net Zn absorption was reduced, high levels of dietary fiber increased Zn absorption from the large intestine in pigs (Partridge, 1978).

It seems that all segments of the GI tract have the ability to absorb Zn. The capacity of Zn absorption of each segment may depend on the animal species. Additionally, shifts in Zn absorption from one segment to the other may occur with changes in dietary composition.

Minerals Interacting with Zn during Absorption. Transport of Zn from the intestinal lumen into the enterocyte can be impeded by other minerals. Iron and Cd have been shown to inhibit Zn uptake from an open-ended duodenal loop in Fe-deficient mice (Hamilton et al., 1978) An interaction between Zn and Fe was also observed from jejunal segments of Fe-adequate rats. Addition of Zn to the perfusate reduced absorption of Fe by 34% (El-Shobaki and Srour, 1989).

Substantial inhibition exerted by Fe on Zn absorption in men was reported by Solomons and Jacob (1981). The inhibition became more apparent with increasing ratios of dietary Fe to Zn. In a subsequent study, the interaction was shown to involve competition between Fe and Zn at intraluminal and intracellular sites (Solomons et al., 1983).

Copper uptake by intestinal brush border membrane vesicles of rats fed either high levels of Zn, adequate Cu and Zn or Cu-deficient diets was studied by Fischer and L'Abbe (1985). They observed the highest Cu uptake by vesicles of rats fed high Zn. Induction of Cu deficiency did not increase vesicular uptake of Cu. Copper transport across the brush border membrane appeared to only occur by simple diffusion (Fischer and L'Abbe, 1985). High dietary intake of Cu was associated with an increase in plasma Cu levels and Cu and Zn contents of the liver in pigs (Shurson et al, 1990). A concurrent decrease was observed for Fe contents in plasma and liver.

Zinc retention was not different in pigs fed varying levels of Ca (Morgan et al., 1969). In rats, a negative effect of Zn on Ca uptake by intestinal brush border membrane vesicles was only observed at high Zn to Ca ratios (Roth-Bassell and Clydesdale, 1991).

A plausible explanation for the interaction between Zn and Cu can be deducted from the model of Zn absorption (Figure 3) proposed by Richards and Cousins (1975). The model suggests an important role for metallothionein which is synthesized proportionally to dietary Zn intake. Accordingly, rats fed high dietary Zn in the study of Fischer and L'Abbe (1985) may have had increased intestinal metallothionein contents. Metallothionein preferably binds Cu over Zn (Cousins, 1985). Large amounts of intracellular Cu bound to metallothionein apparently had

a stimulatory effect on Cu transport across cytosol-free vesicles. An involvement of active transport mechanisms in Cu was not verified by Fischer and L'Abbe (1985).

Because of the lack of detailed models on mineral absorption, research on mineral interactions has mostly been of a descriptive nature. Nevertheless, it has become clear that Cu and Fe affect Zn absorption. It is not clear, whether both minerals actually interfere with cellular uptake of Zn or whether Cu and Fe interact intraluminally with Zn.

Intrinsic Factors Affecting Zn Absorption. Zinc absorption may be affected by dietary and animal factors. In rat jejunal segments, an excess of unhydrolyzed glucose polymers and slowly absorbed sugars reduces Zn absorption (Wapnir et al., 1989). Absorption of Zn is also affected by protein source (Miller and Jensen, 1966; O'Dell et al. 1972) and protein concentration (Hunt and Larson, 1990; Hunt and Johnson, 1992).

The negative association of protein with Zn absorption may be due to other dietary components present in the protein source. Contamination of whole cereal protein with dietary phytase proved to be the cause of an allegedly higher Zn absorption from diets containing animal protein (Harmuth-Hoene and Meuser, 1987). Phytate and also fiber (Davies and Reid, 1979) are two well known intrinsic factors which have been shown to negatively affect the absorption of Zn, Ca and P (Simons et al., 1990).

Animal factors also may interfere with Zn absorption in order to maintain Zn homeostasis (Figure 2). A decrease in the plasma Zn level was observed by Sturniolo et al. (1991) after selective inhibition of gastric acid secretion in men. Their explanation was that a more alkaline environment in the stomach induces the

formation of insoluble Zn compounds which cannot be absorbed further down the GI-tract (Sturniolo et al., 1991).

The uptake of Zn has been shown to be dependent on the bodies need for Zn. The capacity for Zn transport (V_{max}) by intestinal brush border membrane vesicles of rats fed adequate levels of Zn was twice as low as the V_{max} observed with vesicles of Zn depleted rats (Menard and Cousins, 1983). The affinity for Zn (K_m) was not affected by previous dietary Zn intake. Apparently, the high transport rate was due only to an increase in number of receptors.

Many dietary factors interact with Zn absorption. To maintain Zn homeostasis, the animal regulates Zn absorption. Its ability to exert control over the homeostatic mechanisms depends on age, sex, Zn status and other intrinsic animal factors.

Bioavailability of Zinc

The term "bioavailability" is generally used to describe the properties of absorption and utilization of nutrients (O'Dell, 1984). Only absorbed nutrients which participate in the biological processes of the animal after absorption are considered utilized, and thus bioavailable.

Considerations when Determining Zn Availability. The use of Zn depleted animals as part of the design of an availability study may be very important. In a study conducted by Hallmans et al. (1987), bioavailability of Zn from one test food was estimated using two groups of rats with different needs for Zn to maintain Zn homeostasis. To increase the biological need for Zn, a solution containing amino acids was intraperitoneally injected in rats of the treatment group. The controls

were injected with physiological saline. Rats receiving the amino acid solution injection had a 40 % increase in Zn absorption from the test food (Hallmans et al., 1987).

In addition to using Zn depleted animals, the level of Zn in diets used for the process of Zn repletion has to be carefully selected. If the levels of Zn are too low, the animal may not be able to induce biological processes which stimulate feed intake. Conversely, Zn repletion may occur too fast if levels of Zn are too high. After repleting its body stores, the animal maintains Zn homeostasis by decreasing Zn absorption, increasing Zn excretion and sequestering of Zn in tissues (Aggett, 1991). The inability to take measurements during the process of Zn repletion may result in false estimates of Zn availability from diets or mineral sources. Thus, it seems appropriate to initially conduct a dose response experiment to determine an optimum level of Zn for the repletion diets. The level of Zn used should enable the animal to restore biological processes. Furthermore, it should allow time for measurements indicative of effectiveness of Zn repletion.

Effectiveness and duration of Zn repletion can be determined by monitoring performance, repetitive measurement of serum or plasma levels of Zn, metallothionein, and Zn metalloenzyme activities (Miller et al., 1969; Prasad et al., 1971). Furthermore, the same parameters can be determined in tissues containing sensitive Zn pools at one or more time-points during Zn repletion. Each of these measurements or several of them together may be referred to as a Zn bioassay (Wedekind and Baker, 1990).

When interpreting the results of a bioassay, Zn can only be considered bioavailable after proven to be both absorbed and utilized, i.e., contribute to biological functions. Zinc could have been absorbed and be present in serum and

body fluids, but it may not have been utilized. Thus, activities of Zn metalloenzymes or levels of metallothionein in body fluids and tissues may be the best indicators to determine Zn availability from diets and mineral sources.

Complexes and Chelates of Zinc

Metal complexes are compounds of a central metal atom together with ligands which contain at least one ligand atom with a free electron pair. Proteins and carbohydrates including their derivatives, lipids, and many synthetic compounds which contain an O, S or N atom may function as ligand (Kratzer and Vohra, 1986). The number of ligands that bind the metal atom usually exceeds the number expected under valency considerations (Smith, 1990). Bonding of the ligand to the metal occurs through donation of the free electron pair of the ligand atom to the metal atom which acts as an electron acceptor (Figure 4). This type of bond is referred to as coordination or dative bond. Coordination bonds are mostly formed between the transitional elements and the electronegative atoms oxygen and nitrogen (Kratzer and Vohra, 1986). Coordination spheres of some known Zn sites in enzymes are shown in Figure 4.

A metal chelate is a special form of a metal complex. Instead of one ligand atom, two or more atoms of the ligand donate their electron pairs to the metal in the formation of coordination bonds. The chemical ring structure of a chelate resembles a pincer-like claw, for which the Greek word is "Che'le" (Figure 4). Formation of metal complexes and chelates are reversible processes. A continuous exchange of ligands occurs with a change in intraluminal or intracellular

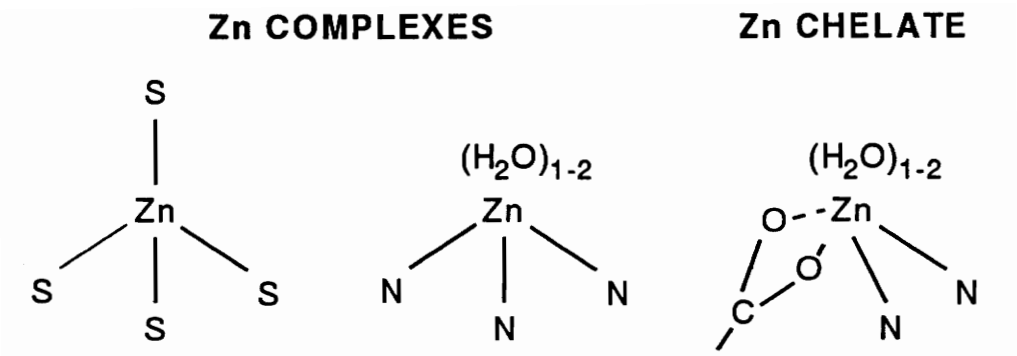

Figure 4. Complexes and chelates of zinc as found at some known Zn sites of Zn containing proteins. Right, structure site of metallothionein (in part) and alcohol dehydrogenase. Center, active site of carbonic anhydrase. Left, active site of carboxypeptidases

environment. The ligands forming the chemically most stable complexes or chelates are preferred (Kratzer and Vohra, 1986).

Bioavailability and Absorption of Complexes and Chelates of Zn. An improvement in Zn availability from the complex Zn methionine compared with $ZnSO_4$ was observed after measuring levels of Zn in the tibia of chicks (Wedekind et al., 1992). In other studies, Zn availability of Zn methionine and an inorganic salt, as assessed by performance and serum Zn levels, were not different in pigs (Kornegay and Thomas, 1975; Hill et al., 1986) and heifers (Spears, 1989). Addition of picolinic acid, a tryptophan derivative in combination with Zn methionine to pig diets also did not improve Zn availability (Hill et al., 1986).

Although apparent Zn absorption from Zn methionine was not different from ZnO, Spears (1989) did observe an increase in Zn retention of lambs fed Zn methionine. The increase in Zn retention was caused by a slight reduction in urinary Zn excretion. A higher Zn retention with no difference in Zn absorption (Spears, 1989) suggests that Zn from Zn methionine is absorbed more slowly than Zn of ZnO.

Addition of Zn methionine to diets of chicks fed adequate amounts of ZnO slightly increased the content of Zn in the pancreas compared with chicks fed extra ZnO (Pimentel et al., 1991). Growth, and concentrations of Zn, Cu and Fe in tibiotarsus and liver were not affected by the Zn source (Pimentel et al., 1991). Carcass quality of steers was improved when Zn methionine was supplemented to the diet instead of ZnO (Greene et al., 1988). The better carcass quality was not associated with improved performance.

Picolinic acid and Zn were evaluated as a Zn-complex by Roth and Kirchgessner (1985). They observed no differences in body gain, serum Zn level,

and content of Zn in testes, femur and whole body of rats fed Zn picolinate, Zn citrate or $ZnSO_4$.

In humans, a 25% increase in serum Zn level was observed after ingestion of Zn as a Zn histidine complex compared with $ZnSO_4$ (Schölmerich et al., 1987). The apparent higher absorption of Zn histidine, however, was associated with increased urinary Zn excretion. Performance of grower pigs was not improved by supplementing 1% histidine or 289 ppm EDTA to Zn adequate diets (Dahmer et al., 1972; Owen et al., 1973). Interestingly, inclusion of histidine appeared to alleviate skin lesions of the Zn deficient pigs used by Dahmer et al. (1972). This may indicate that dietary histidine did improve Zn absorption. Alternatively, a high level of absorbed histidine may have stimulated the healing process independent of Zn.

Dietary Organic Ligands Affect Zn Absorption. The influence of a variety of amino acids and their chemical homologues on Zn uptake from perfused jejunal, ileal and colonic segments of rats was studied by Wapnir and Stiel (1986). In the small intestine, perfusion with tryptophan, histidine, cysteine and proline achieved a higher Zn uptake than perfusion with their respective homologues tryptophol, imidazole, N-acetyl-L-cysteine and pyroglutamate. Zinc uptake was not differently stimulated by the amino acids. Substantial uptake of Zn from the colon was only observed with imidazole, which may be explained by the structural affinity of imidazole for Zn (Wapnir and Stiel, 1986).

The relationship between several inorganic and organic ligands of Zn and Zn absorption in rats was studied by Seal and Heaton (1983) and Giroux and Prakash (1977). Salient features of their results are shown in Figure 5. Uptake of Zn by duodenal and ileal sacs was highest when the organic ligand 2-picolinic acid was

included into the incubation buffer (Seal and Heaton, 1983). Of the inorganic ligands tested, sulfate proved to be the most effective in enhancing Zn uptake. Compared to sulfate, the amino acids histidine and cysteine did improve Zn uptake from the ileal, but not from the duodenal sac. In general, Zn uptake was higher from the duodenal than from the ileal sac.

As a part of their study, Seal and Heaton (1983) evaluated the most promising ligands in vivo in a metabolic balance study. The ligand 2-picolinic acid did not improve Zn retention as expected, primarily because of increased urinary Zn excretion. Plasma Zn levels also were not different among the groups fed $ZnCl_2$, $ZnSO_4$ and $ZnCl_2$ in combination with 2-picolinic acid (Seal and Heaton, 1983).

Acidic ligands proved to be the most effective in increasing levels of serum Zn 1 h after feeding (Giroux and Prakash, 1977). The ligand and $ZnSO_4$ mixtures (10 ppm Zn) were fed with a stomach tube to rats after a 24-h fast. A 1:1 mixture of phytate and $ZnSO_4$ reduced serum Zn about 3.5 times compared with the control $ZnSO_4$. Of the amino acids tested, a 2:1 mixture of glycine and $ZnSO_4$ was most effective. However, increases in serum Zn levels observed with the amino acids lysine, histidine and cysteine were only slightly lower. Serum Zn levels, measured 4 h after feeding, returned to the $ZnSO_4$ control value for most amino acid and $ZnSO_4$ mixtures. Differences in levels of serum Zn observed with phytate and several acidic ligands at 1 h after feeding, were still present 4 h after feeding (Giroux and Prakash, 1977).

Complexes and Chelates of Other Trace Minerals. In addition to Zn, bioavailability and absorption of several complexes and chelates of Fe, Cu, and Mn have been studied. Iron availability of complexes was assessed by Miller et al.

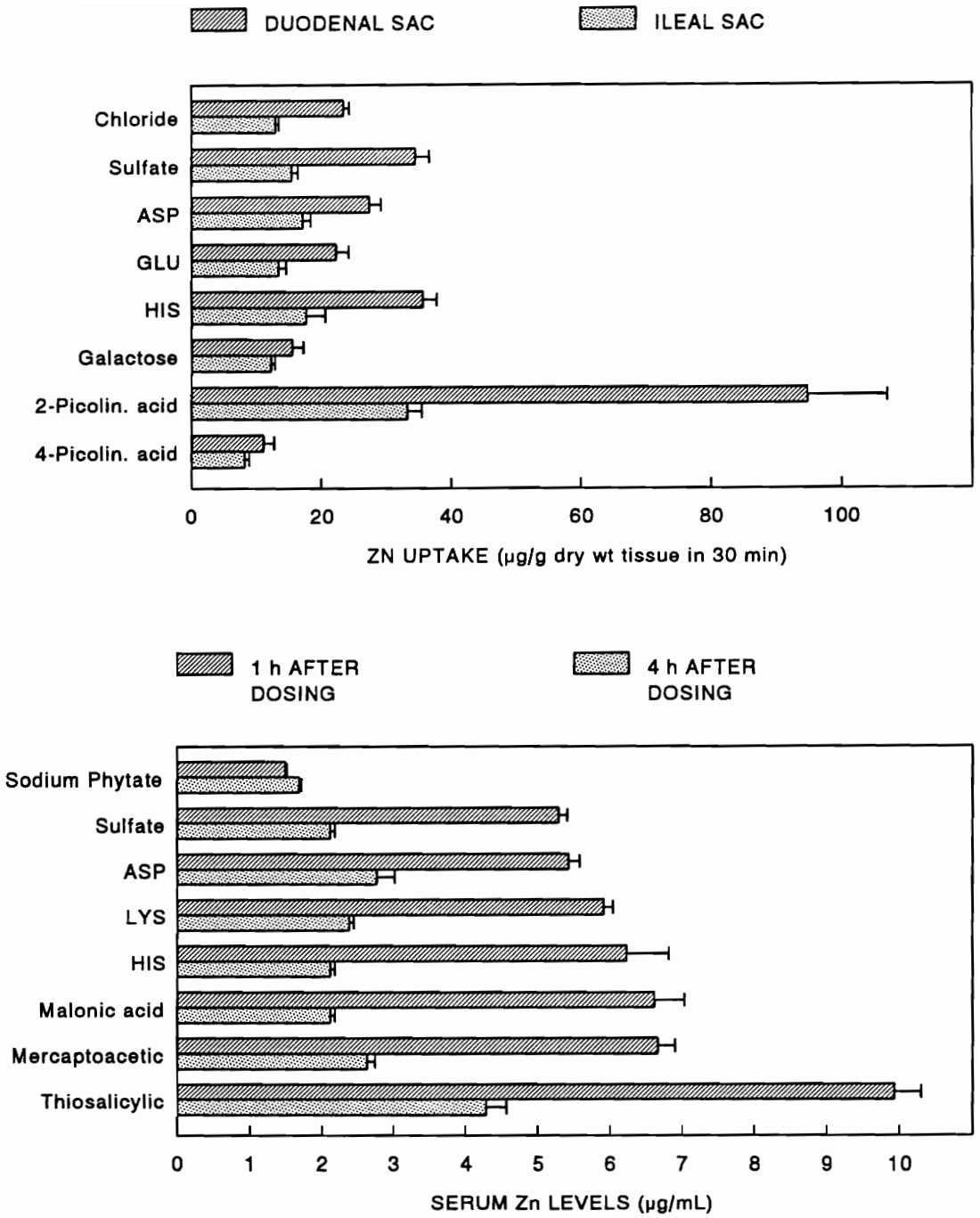

Figure 5. Absorption of Zn as influenced by dietary ligands. Zinc uptake (top) by duodenal and ileal everted gut sacs of rats (Seal and Heaton, 1983). Serum Zn levels (bottom) measured in rats 1 and 4 h after oral dosage of Zn and ligand (Giroux and Prakash, 1977)

(1981a) using hemoglobin regeneration levels of anemic pigs. The amount of available Fe supplied by ferric choline citrate and ferric copper cobalt choline citrate was 140%, relative to $FeSO_4$ (Miller et al., 1981a). Measuring hemoglobin levels of nursing pigs, Brady et al. (1978) did not observe a difference after supplementing 500 ppm Fe, either as Fe proteinate or $FeSO_4$, to the diets of their dams.

Based upon the accumulation of Cu in chicken livers, Baker et al. (1991) reported that the complex Cu lysine provided the same availability of Cu as $CuSO_4$. A Cu proteinate, compared with $CuSO_4$ in steers, did not increase levels of Cu in plasma or liver (Wittenberg et al., 1990). Copper supplementation with an organic Cu chelate provided no additional benefit to performance of nursery and growing pigs compared with pigs fed diets supplemented with Cu EDTA or $CuSO_4$ (Stansbury et al., 1990).

Supplementation of a Mn proteinate did not increase bone Mn compared with $MnSO_4$ in chicks during a 14-d accumulation period (Baker and Halpin, 1987). An improvement in Mn availability with Mn methionine was reported by Fly et al. (1989). However, their conclusions were based on regression analysis with very few observations at extremely different supplemental levels of MnO (126 and 1,256 ppm) and Mn methionine (186 and 1,860 ppm).

In a 2-yr study with beef cows and calves, Spears and Kegley (1991) showed only a modest advantage of overall performance attributable to the use of Zn methionine and Mn methionine instead of ZnO and MnO.

Data on mineral availability of organic metal complexes and chelates are inconclusive. Interpretation is difficult because of extreme variations among studies. Firstly, many of the studies evaluated different complexes or chelates.

Secondly, measurements used to determine the effectiveness of mineral absorption differed from study to study. Thirdly, mineral availability of the inorganic mineral source used as the control may have influenced the outcome of the comparison. For example, based on tibia Zn, Wedekind and Baker (1990) estimated a 61.2% availability of Zn from ZnO relative to $ZnSO_4$. Measuring levels of serum Zn, Miller et al. (1981b) observed a 30% greater Zn availability from metallic Zn dust than from ZnO.

Further problems may arise with translation of in vitro results to practice. This was well illustrated by the study of Seal and Heaton (1983). The ligand 2-picolinic acid was promising in vitro, but was without effect when tested in a metabolism trial.

Mode of Action of Complexes and Chelates of Zn. Organic ligands may affect Zn absorption by effectively competing for Zn with non-absorbable dietary constituents. Alternatively, organic ligands may actually participate in the poorly understood process of Zn absorption.

Recently, it has been shown that dietary additives can reduce binding of Zn to dietary constituents which can not be absorbed. A commercially produced phytase dramatically improved the bioavailability of Ca and P presumably by releasing the minerals from phytic acid (Simons et al., 1990). Zinc complexes or chelates which are thermodynamically favored over the Zn phytate complex are likely to be as effective as phytase in increasing luminal Zn availability.

To assume that dietary Zn complexes and chelates promote Zn absorption is far more interesting. Only a few studies have investigated this hypothesis.

The uptake of Zn, determined with everted duodenal sacs of pigs, was not different among $ZnSO_4$, Zn methionine and Zn lysine (Hill et al., 1987). With the

addition of 5 M picolinic acid to the buffer containing organic Zn forms, a reduction in Zn uptake was observed for each of the three Zn sources (Hill et al., 1987). Uptake, mucosal retention and absorption of Zn from $ZnCl_2$, $ZnCl_2$ with methionine, Zn methionine complex and $ZnCl_2$ with EDTA were studied by Hempe and Cousins (1989) with the aid of ligated rat duodenal loops. After 60 min of incubation, Zn uptake and absorption were reduced for Zn methionine and the Zn EDTA mixture. It was suggested that the low Zn absorption was associated with reduced binding of Zn to an unidentified low molecular weight protein present in the mucosa (Hempe and Cousins, 1989). Recently, Cousins and Lee-Ambrose (1992) proposed a role for the presently identified Zn protein in transcellular transport (Figure 3). It may be that Zn uptake from Zn methionine complex and Zn EDTA is reduced due to their inability to donate Zn to the transport protein.

Kinetic studies using pig intestinal brush border membrane vesicles were performed to evaluate the effect of several ligands on Zn uptake at low (5 μM) concentrations of Zn (Turnbull et al., 1990). Vesicular Zn uptake was decreased when concentrations of citrate, phytate and picolinate were increased. The ligands folate, histidine, glucose and polyose did not affect Zn uptake.

Efflux of Zn from liposomes increased after addition of picolinic acid to the buffer (Aggett et al., 1989). With picolinic acid trapped with Zn inside the liposome a decrease in Zn efflux was observed. A similar pattern of efflux was observed with other minerals, suggesting that the chelating properties of picolinic acid are non-specific.

The involvement of a highly negative anion, $Zn(SCN)_4^{2-}$ which considerably augmented vesicular Zn uptake in the absence of a membrane potential has been reported (Tacnet et al., 1990). A beneficial effect on Zn uptake of negatively

charged ligands was also reported by Giroux and Prakash (1977). They observed the highest increase in levels of serum Zn with mixtures of ligands and $ZnSO_4$ that formed negatively charged compounds.

It is now clear that the ligand, inorganic or organic, does have an effect on bioavailability of Zn. A consistent positive effect, however, has not been reported for any of the ligands studied.

A critical review of the assumptions made in most studies reveals the following. Most researchers examined absorption of Zn complexes and Zn chelates within the small intestine, neglecting stomach and large intestine. Granted, reportedly the small intestine is the site from which Zn absorption primarily occurs. Moreover, many of the ligands tested in complexes and chelates of Zn are believed to be largely absorbed in the small intestine. However, what if the complex or chelate of Zn is absorbed as an entity in a GI-segment other than the small intestine?

To examine this possibility, the site(s) of absorption of Zn has to be (re)evaluated for inorganic Zn salts, Zn complexes and Zn chelates. Initially, the whole GI-tract should be considered. The newly obtained information may suggest involvement of the organic Zn products in the current model of Zn absorption. Alternatively, more information on the site(s) of Zn absorption may suggest mechanisms that are presently not considered. Either mechanisms specific for the organic complex or chelate of Zn, or mechanisms specific for the ligand.

Summary

Zinc is one of the trace elements essential for the normal functioning of living organisms. The trace element Zn is essential as part of organic compounds which play regulatory roles in biological processes. Although absorption of Zn has been extensively studied, the mechanisms involved in the different processes are poorly understood. Interactions of Zn with other minerals, and dietary and animal factors have been recognized, but their mode of action remains to be elucidated. Dietary complexes and chelates of Zn have been suggested to supply more bioavailable Zn than inorganic Zn salts. Thus far, data have been inconclusive. Basic research which investigates possible mechanisms involved in Zn absorption from complexes and chelates of Zn is warranted.

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CHAPTER III

Evaluating Novel Indicators for Assessing Zinc Status during Experimental Zn Depletion of Pigs

Abstract

In three experiments, pigs were fed an isolated soy protein, semipurified diet with no added Zn to evaluate conventional and novel indicators for assessing Zn status during experimental Zn depletion. The low Zn depletion diet provided 17 ppm Zn. Control pigs were pair-fed the low Zn diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) to assess deficiency symptoms specific for Zn. Serum Zn concentrations and serum alkaline phosphatase (ALP) activity of pigs fed the low Zn diet were reduced ($P < .01$) compared with the control pigs, respectively, after 7 and 14 d. Furthermore, ADG and gain to feed ratio of pigs fed the low Zn diet were reduced ($P < .05$) after 14 d. At the end of Zn depletion in Exp. 2, serum mitogenic activity and pituitary RNA content were lower ($P < .05$) for pigs fed the low Zn diet compared with the $ZnSO_4$ and ZnAAC controls. Moreover, the growth hormone (GH) mRNA fraction of RNA tended to be reduced ($P < .10$) for the low Zn group. In Exp. 2, Zn concentrations in liver, pancreas, kidney, brain and small intestinal tissues of pigs fed the low Zn diet were lower ($P < .01$) compared with the $ZnSO_4$ and ZnAAC control groups. Parakeratosis evolved in all pigs fed the low Zn diet but in none of the pair-fed controls. In conclusion, serum mitogenic activity and pituitary RNA and GH mRNA content were sensitive indicators for assessing Zn status in fast growing pigs.

(Key words: growth hormone mRNA, growth factors, zinc deficiency, pigs, semipurified diet)

Introduction

Many measurements have been proposed as indicators for the assessment of Zn status in humans and animals. In humans, sensitivity of the indicator is a primary selection criteria because clinically it is important to detect early onsets of Zn deficiency (Aggett, 1991; King, 1990). In animals, the same indicators are often used to determine differences in availability of Zn from nutritional sources (Smith et al., 1961; Hill et al., 1986; Wedekind et al., 1992). Although less critical than in clinical nutrition, indicators with a high degree of sensitivity would be preferred to allow detection of small differences in Zn availability. To date, Zn concentrations and Zn metalloenzyme activities in body fluids and tissues are most frequently used for assessing Zn status (Miller et al., 1969; Prasad et al., 1971; Roth and Kirchgessner, 1974).

One of the first signs observed during Zn deficiency is lack of growth. Recently, decreases in blood levels of insulin like growth factor (IGF)-1 and insulin have been associated with growth retardation during Zn deficiency of rats (Cossack, 1986; Dørup et al., 1991). Normally, serum IGF-1 levels are maintained by secretions of hepatic IGF-1. Synthesis of IGF-1, in turn, is regulated by pituitary growth hormone (GH) through stimulation of transcription and(or) maintenance of hepatic IGF-1 mRNA stability (Mathews et al., 1986; Roberts et al., 1986).

In the present study, serum mitogenic activity and pituitary growth hormone gene expression were evaluated, in addition to serum and tissue Zn concentrations

and serum ALP-activity, as indicators for assessing Zn status during experimental Zn depletion of pigs.

Experimental Procedures

Animals, Diets, and Housing. The study consisted of three experiments. In Exp. 1, 48 crossbred pigs, BW 6.8 $\pm$ .1 kg and age 25.0 $\pm$ .4 d, were used (Figure 1). During an adjustment period of 5 d, all pigs were fed a corn soybean meal diet with 20% dried whey (30 ppm Zn) with no supplemental Zn added. After the adjustment period, 40 pigs were given ad libitum access to a semipurified diet (Table 1) that met all NRC suggested nutrient requirements, except Zn (NRC, 1988). The low Zn depletion diet provided 17 ppm Zn. Concurrently, eight control pigs (four per group) were fed the low Zn diet supplemented with 45 ppm Zn as $ZnSO_4.7H_2O$ or as Zn amino acid chelate (Albion Laboratories, Cadco Inc., Des Moines, IA). The amount of feed offered to the eight control pigs was adjusted on a metabolic BW basis to the mean feed consumption of the 40 pigs fed the low Zn diet.

At the end of the Zn depletion period, six pigs, randomly selected from the low Zn group, were sacrificed by intravenous injection of a lethal dose (.25 mL per kg BW) of sodium pentobarbital (Anthony Products Co., Arcadia, CA) followed by exsanguination. Furthermore, a necropsy was performed on three randomly selected pigs of the low Zn group, and seven Zn depleted pigs were not used. The remaining 24 Zn depleted and the eight control pigs were used in a Zn repletion study described in Chapters IV and V.

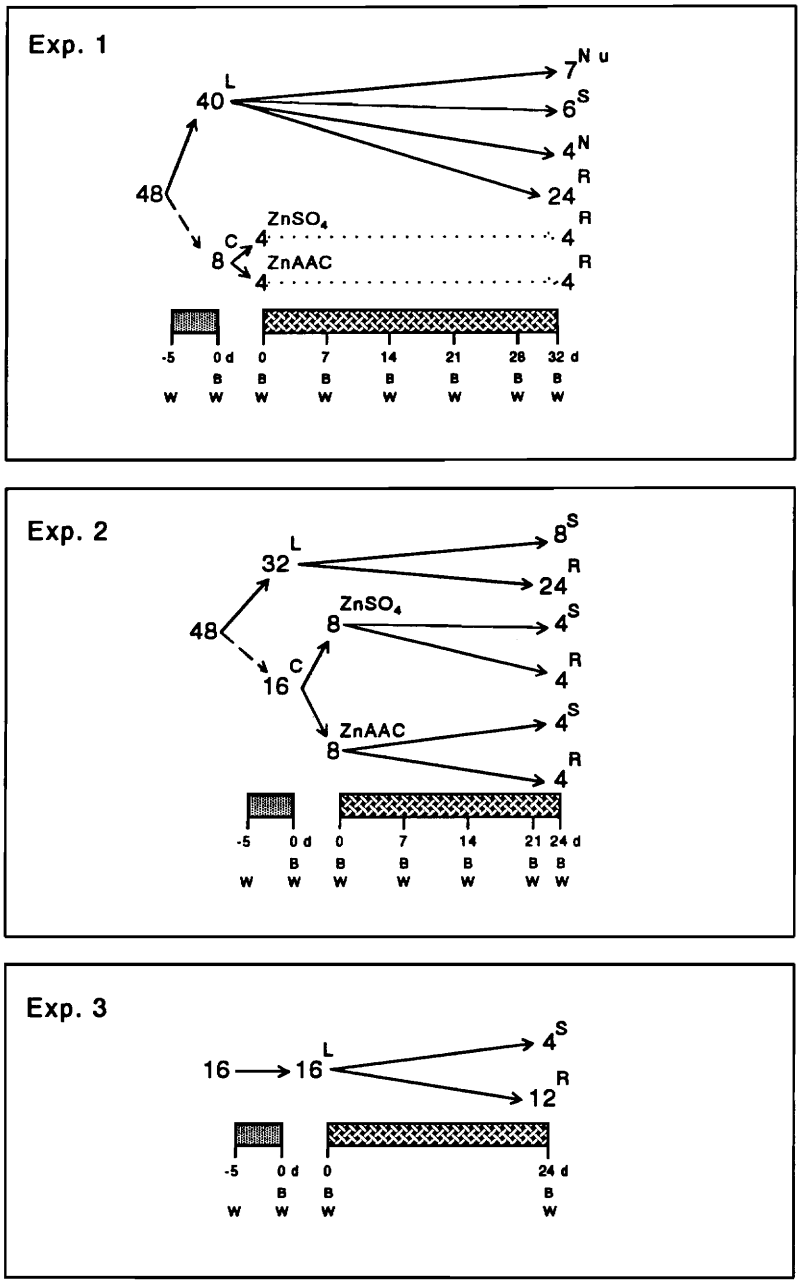

Figure 1. Schematic overview of experimental design and measurements of Exp. 1, 2 and 3. Legend: L = low Zn, C = control, ZnAAC = Zn amino acid chelate, N u = not used, S= sacrificed, N = necropsied, R = used in repletion study, B = blood sampled, and W = weighed

adjustment period Zn depletion period

Table 1. Composition of the semipurified diet

Ingredient	(g per kg diet)
Corn starch[a]	336.6
Dextrose[b]	300
Isolated soy protein[c]	200
Corn oil	60
Cellulose[d]	30
Dynafos[e]	29
Mineral mix[f]	16.6
Dyna-K[e]	14.4
$CaCO_3$	5.8
Vitamin mix[g]	4
Dynamate[e]	3.6

[a]Cargill Inc., Minneapolis, MN
[b]Cerelose Dextrose 2001, Corn Products, Summit-Argo, IL
[c]PP500E, Protein Technologies International, St. Louis, MO
[d]Purified powder cellulose BH200, International Filler Co., North Tonawanda, NY
[e]Dynafos contained (minimum) 20% Ca, 18.5% P, and (maximum) .185% F; Dynamate contained (minimum) 22% S, 18% K, and 11% Mg; Dyna-K contained (minimum) 96.5% KCl and 50% K. Pitman-Moore Inc., Mundelein, IL
[f]Provided the following sources of minerals in g per kg of diet: $FeSO_4.7H_2O$, .25; $CuSO_4.5H_2O$, .024; $MnSO_4.H_2O$, .012; KIO_3, .00024; Na_2SeO_3, .00066; dextrose, 15.3
[g]Provided the following amounts per kg of diet: vitamin A, 4,950 IU; vitamin D_3, 660 IU; vitamin E, 33 IU; vitamin K, 6 mg; riboflavin, 3.3 mg; niacin, 35.2 mg; pantothenic acid, 19.8 mg; vitamin B_{12}, .022 mg; choline, 1,184 mg; thiamin, 2.6 mg; vitamin B_6, 3 mg; biotin, .4 mg; folacin, 2mg; D,L-methionine, 1,200 mg

In Exp. 2, 48 pigs, BW 5.3 $\pm$ .1 kg and age 20.2 $\pm$ .1 d, were used (Figure 1). After the 5-d adjustment period, 32 pigs were assigned to a similar low Zn diet as used in Exp. 1. Concurrently, 16 control pigs (eight per group) were assigned to the low Zn diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC). At the end of the Zn depletion period, eight Zn depleted pigs and four pigs of each control group were randomly selected and then sacrificed (Figure 1). The remaining 24 Zn depleted pigs and eight control pigs were used in a Zn repletion study described in Chapter V.

In Exp. 3, 16 crossbred pigs, BW 6.8 $\pm$ .3 kg and age 23.3 $\pm$ .1 d, were assigned to a similar low Zn diet as previously used in Exp. 1 and 2 (Figure 1). At the end of Zn depletion, four randomly selected pigs were sacrificed. The remaining twelve pigs were used in a Zn repletion study described in Chapters IV and V.

Pigs were housed in 1.5 m x .7 m stainless steel pens (four pigs per pen) in a room equipped with temperature and ventilation control. Ambient temperature was maintained at 29.5°C during the adjustment period. Thereafter, temperature was reduced by approximately 2°C weekly to a low of 23°C. A 12-h light-dark cycle was maintained.

Performance, Blood, and Tissue Collection. Body weight and feed intake were monitored weekly during the Zn depletion periods in Exp. 1 and 2 (Figure 1). In Exp. 3, BW was determined at the start and end of the 24-d Zn depletion period. Feed intake was monitored throughout the entire period. Blood samples were taken from the anterior vena cava on the days of weighing. Immediately after collection, blood samples were refrigerated (4°C) for 24 h to allow formation of a blood clot prior to centrifugation. After centrifugation at 625 x g for 10 min, serum

was removed and sampled. Two samples were stored in a refrigerator (4°C) for the analyses of serum Zn concentrations and ALP-activity. A third sample was frozen (-85°C) for later analyses of total serum mitogenic activity and total serum protein concentrations.

All pigs were sacrificed within 2 to 4 h after offering the meal. In all experiments, samples of liver and kidney were collected from each sacrificed pig. Moreover, samples of pancreas, brain and the intact pituitary were collected in Exp. 2. Additionally, the small intestine was excised in Exp. 1 and 2 and the stomach in Exp. 3. The excised small intestine was ligated into three segments: (1) proximal small intestine (one-third distal of pylorus), (2) medial small intestine (one-third distal of pylorus to one-third proximal of ileo-cecal valve), and (3) distal small intestine (one-third proximal of ileo-cecal valve). In Exp. 1, mucosal scrapings were collected from each segment of the small intestine following the procedures described by Wilson and Webb (1990). Each of the small intestinal segments and the stomach segment were flushed three times using physiological saline in Exp. 2 and 3, respectively.

Pituitaries were weighed and collected in 12 x 75 mm polypropylene tubes, immediately flash frozen in liquid N_2 and stored on dry ice (-75°C). Other tissues were weighed, collected in plastic ziploc bags and stored on ice (4°C). Afterwards, pituitaries were stored at -85°C, and tissues were frozen (-20°C) for later analyses of tissue mineral concentrations.

Analyses of Serum and Tissues. In all experiments, serum Zn concentrations were determined using flame atomic absorption spectrophotometry (Perkin Elmer 5100, Cornwalk, CT). Serum alkaline phosphatase (ALP) activity (Sigma Procedure 245) was determined using a Autoanalyzer (Centrifichem 500, Union Carbide, New

York, NY) in Exp. 1, and a computer controlled vertical photometer (Titertek Multiskan MCC/340, Flow Laboratories, McLean, VA) in Exp. 2 and 3. At the end of the 32-d Zn depletion period in Exp. 1, five serum samples of Zn depleted pigs were randomly selected for serum chemical profile analyses using an Autoanalyzer (Kodak Ektachem 700, Eastman Kodak, New York, NY)

In Exp. 2, total serum mitogenic activity, analyzed by a MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) cell proliferation assay using BC_3H_1 myogenic cells, was determined (Zhou et al., in preparation). Additionally, total serum protein concentrations were determined using a Bicinchoninic Acid assay (Pierce BCA Protein Assay Kit 23225, Rockford, IL).

Following an overnight thaw at room temperature, liver, kidney, brain, small intestine and intestinal mucosal scrapings were homogenized using an Osterizer blender. Moreover the pancreas was homogenized using a Polytron homogenizer (PT10/35, Brinkman Instruments, Westberg, NY). The homogenates were analyzed for Zn, Cu and Fe content using flame atomic absorption spectrophotometry following wet digestion with HNO_3 and $HClO_4$ (AOAC, 1990). Dry matter content was determined on homogenates by drying for 48 h in a forced vent oven (105°C).

Pituitary RNA was isolated by acid guanidinium thiocyanate-phenol-chloroform extraction (Meltzer et al., 1990). The OD_{260} and OD_{280} of the extracted RNA fraction were measured using a UV spectrophotometer (Lambda 3B, Perkin Elmer, Cornwalk, CT). The RNA content of the pituitary was estimated from the OD_{260} reading and the purity of the isolate was evaluated by examining the ratio of OD_{260} to OD_{280} (Sambrook et al., 1989). After size separation of 10 μg RNA using a 1.5 % denaturing agarose gel, the RNA fragments were transferred to a nitrocellulose membrane. Northern blot analysis was carried out with a

synthetic ^{32}P labeled oligonucleotide (18 mer) functioning as probe for porcine GH. A desktop scanner (HP Scanjet IIp, Hewlett Packard, Palo Alto, CA) was used to quantify GH mRNA.

Statistical Procedures. Serum data of Exp. 1 and 2, and tissue, small intestinal and pituitary data in Exp. 2 were analyzed by the GLM procedure of SAS (1988) using pig as the experimental unit. Pen was used as the experimental unit for analysis of performance data. Diet was the main effect of the model. Orthogonal contrasts were used to compare the low Zn and combined control groups, and the pigs fed the $ZnSO_4$ and ZnAAC control diets.

The slopes of the serum Zn and serum ALP-activity data were analyzed by regressing on day, day^2 and on the interactions day x diet and day^2 by diet (SAS, 1988) using the following statistical model:

$$Y_{ij} = \mu + Diet_i + Pig(Diet)_{ij} + b_1Day + b_2Day^2 + b_{1i}Day + b_{2i}Day + e_{ij}$$

where: μ = overall mean

$Diet_i$ = effect of $Diet_i$, i = 1, 2, 3

$Pig(Diet)_{ij}$ = Pig nested within Diet,

j = 1 to 40 for i = 1, 2 and

j = 1 to 4 for i = 3

b_1 = linear coefficient of day

b_2 = quadratic coefficient of day

b_{1i} = coefficient of day by diet interaction

b_{2i} = coefficient of day^2 by diet interaction

e_{ij} = error term.

Both linear and quadratic diet by day sequential sums of squares were determined to allow for inferences regarding the similarity of serum Zn concentration and serum ALP-activity patterns of the low Zn, $ZnSO_4$ and ZnAAC control groups. In this model, the diet effect should be tested against $Pig(Diet)_{ij}$ and the remaining effects against the error term.

Results

Mineral concentrations of the low Zn depletion diet were 17 ppm Zn, 16 ppm Cu and 480 ppm Fe. Including 45 ppm supplemental Zn, the $ZnSO_4$ and ZnAAC control diets contained 64 and 66 ppm Zn, respectively.

Parakeratosis. After approximately 2 wk of Zn depletion, parakeratosis, the classical sign of Zn deficiency, was clearly observed in all pigs fed the low Zn diet (Figure 2). Evolving erythematous macules and papules caused reddening of the ventral abdomen and medial thighs of the Zn depleted pigs. During the latter part of the Zn depletion period, the dermatitis spread in a diffusive pattern and evolved into dry hard crusts. Pruritus and greasiness were minimum to absent. Parakeratosis was primarily observed on the distal limbs, face, ears, tail and ventrum. A histological preparation of a parakeratotic site shows evidence of incomplete keratinazation of dermal epithelial cells (Figure 3). At the end of the Zn depletion period, swollen hock joints were found on some of the Zn depleted pigs. Conversely, skin of pigs fed the control diets supplemented with 45 ppm Zn appeared normal and in a healthy condition. Furthermore, diarrhea was more severe and was more frequently observed in the low Zn group than in the $ZnSO_4$

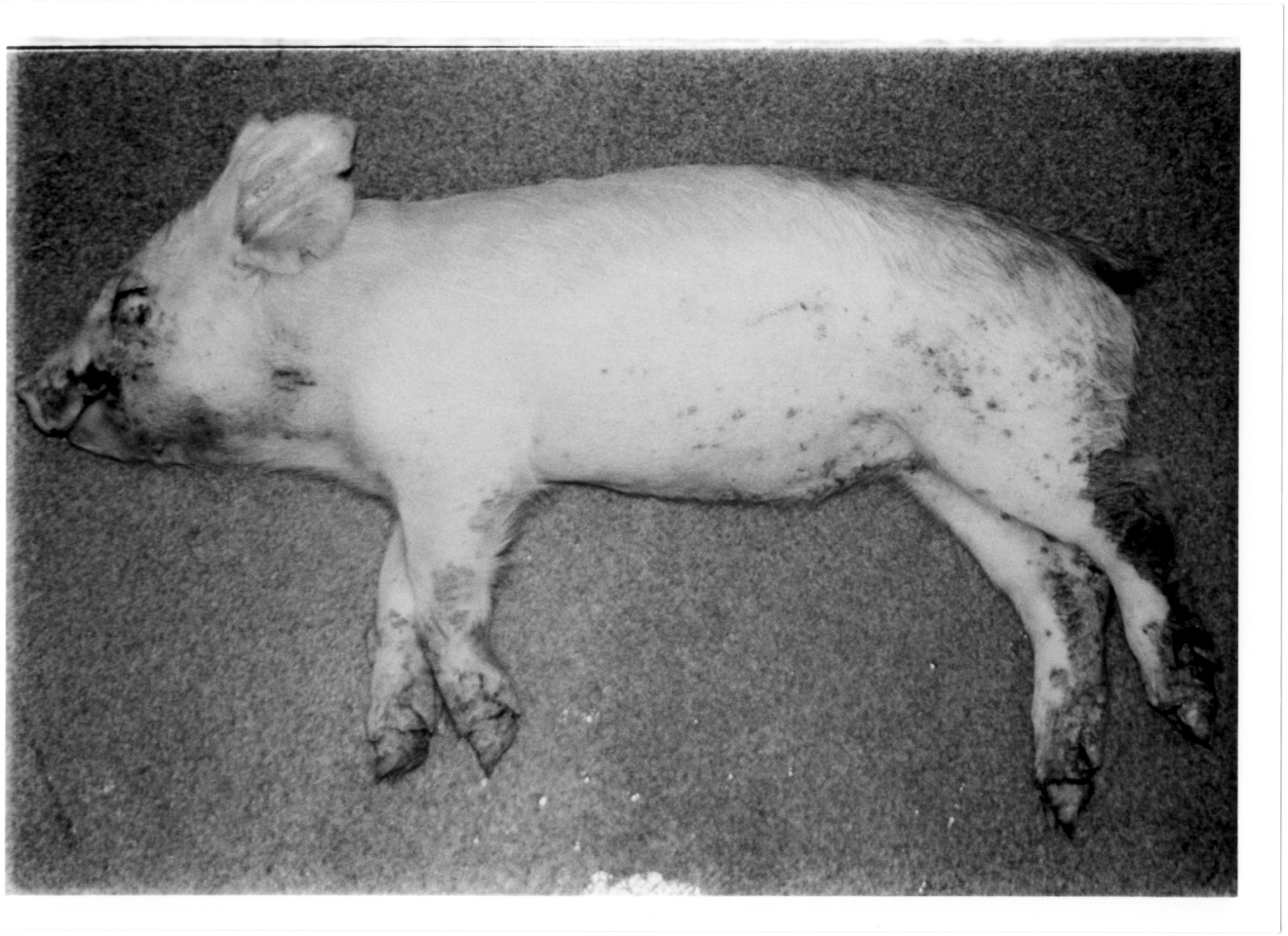

Figure 2. A diffusive pattern of dermatitis is typical of parakeratosis resulting from Zn deficiency

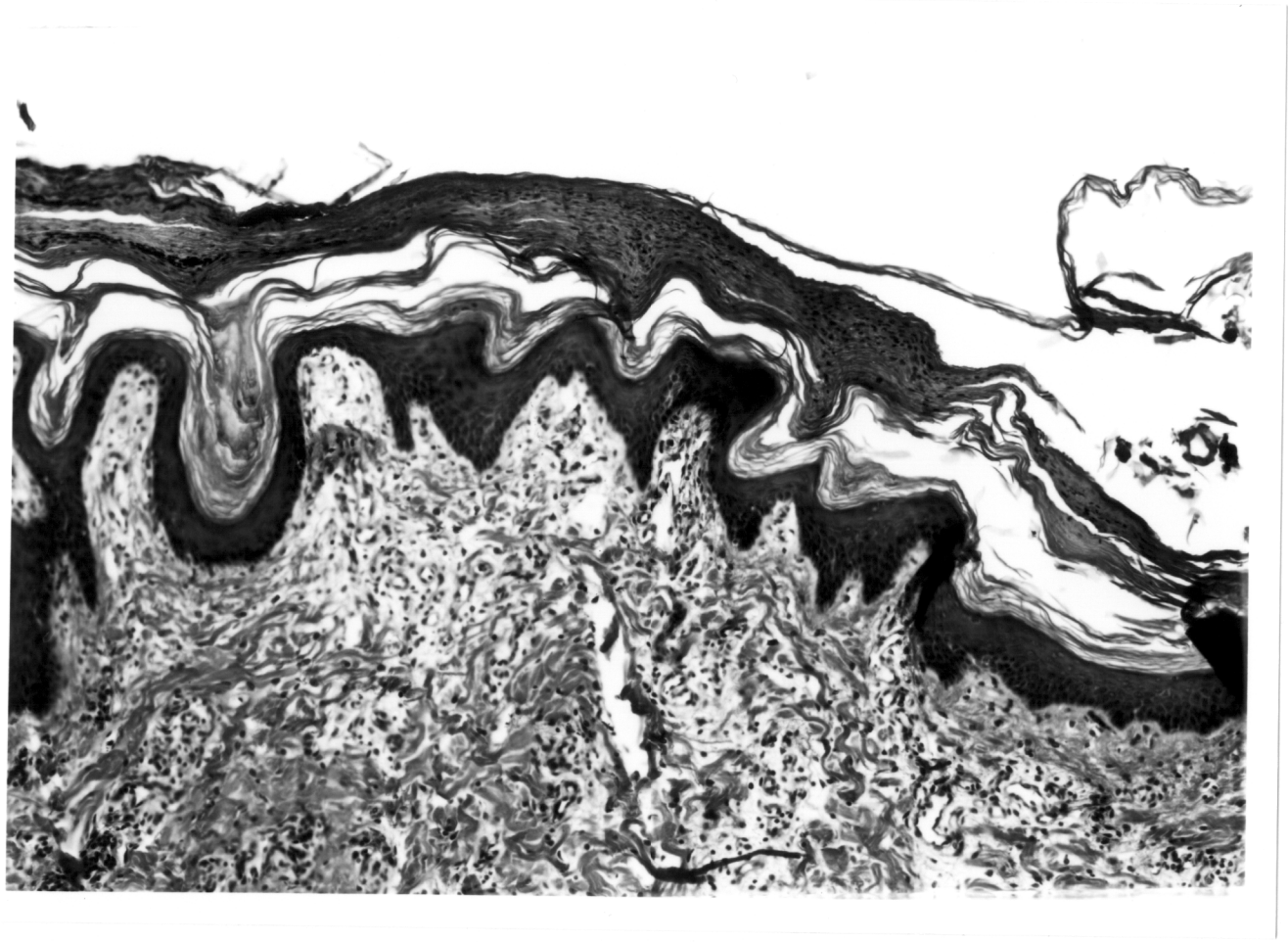

Figure 3. Histological preparation of parakeratotic site shows incomplete keratinazation of the dermis

and ZnAAC control groups. The gross anatomy of the stomach, liver, kidney, pancreas and small intestine of the Zn depleted pigs appeared to be normal.

Serum Characteristics and Performance. It was inferred from the linear ($P < .001$) and interactive ($P < .001$) day by diet effects, that the patterns of serum Zn concentrations and serum ALP-activities were different for the low Zn, $ZnSO_4$ and ZnAAC groups. Serum Zn concentrations and serum ALP-activity of pigs fed the low Zn diet rapidly decreased to d 14 and then plateaued (Figure 4). However, the drop in serum Zn concentrations occurred primarily during the first 7 d, whereas, the drop in serum ALP-activity was steepest from d 8 to 14 of Zn depletion. In Exp. 1, serum Zn levels appeared to decrease for both control groups between d 1 and 14 and then were maintained. In Exp. 2, serum ALP-activity of control pigs gradually decreased after a slight increase during the first 7 d.

In Exp. 1 and 2, serum Zn levels and serum ALP-activities of Zn depleted pigs were on an average 63 % and 85 % lower ($P < .01$), respectively, than the mean values for the $ZnSO_4$ and ZnAAC control pigs. Serum Zn concentrations and serum ALP-activities were similar for the $ZnSO_4$ and ZnAAC control groups.

In Exp. 1, serum protein concentrations and serum P and Mg levels of the Zn depleted pigs appeared to be higher, and serum urea-N and glucose levels lower (Table 2), than those of genetically similar pigs with ad libitum access to a 19 % CP corn soybean meal diet (Schell, 1991). In Exp. 2, serum protein levels were similar for the pigs fed the low Zn and control diets (Figure 5).

After the first 7 d, ADG of pigs fed the low Zn diet was lower ($P < .01$) compared with the limited-fed $ZnSO_4$ and ZnAAC control pigs (Tables 3 and 4). Since feed intake of the low Zn and control groups was similar within each experiment, the reduction in ADG resulted from a smaller ($P < .01$) gain to feed

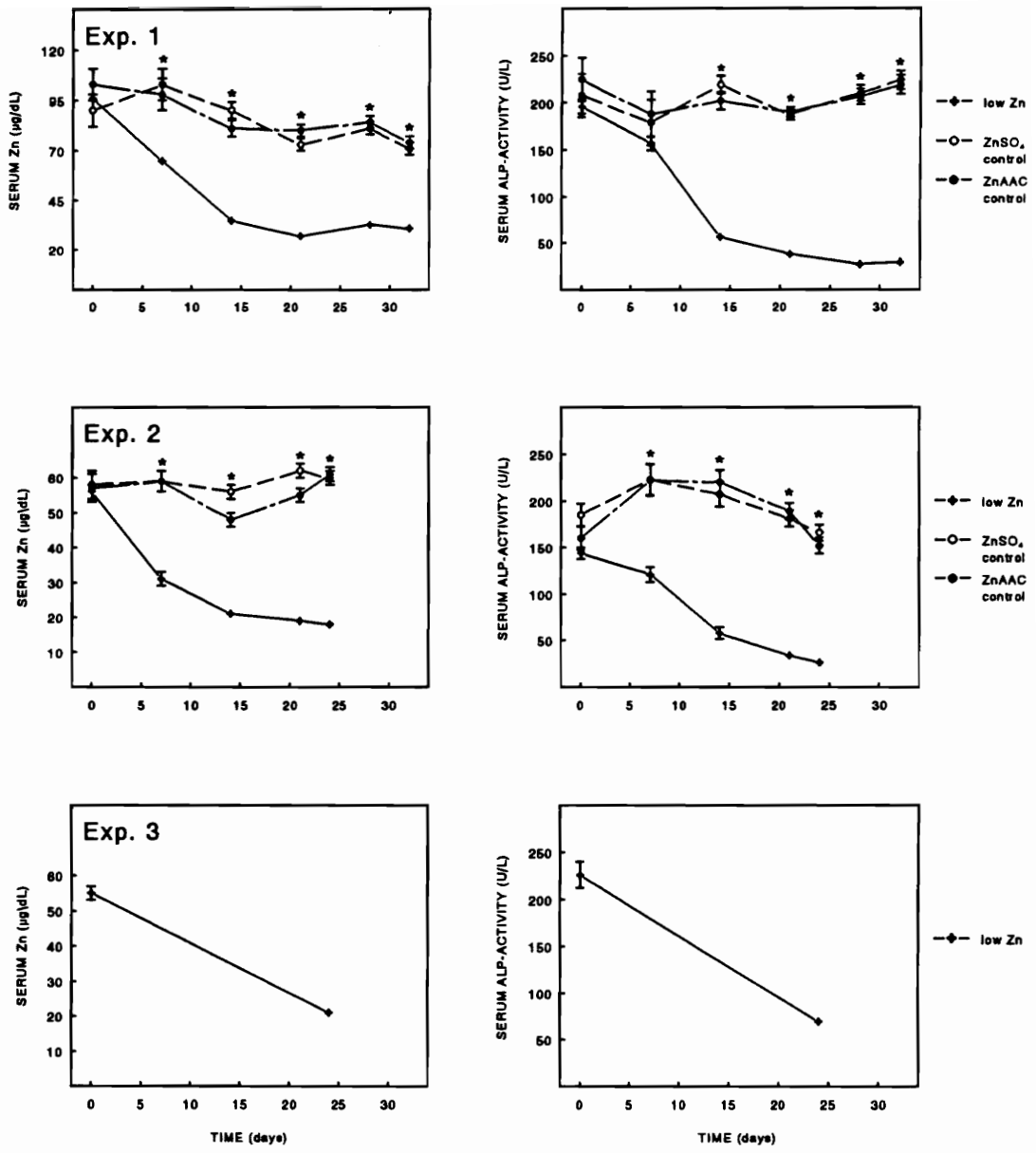

Figure 4. Serum Zn concentrations and serum alkaline phosphatase (ALP) activity of pigs fed the low Zn depletion diet and of pigs fed the $ZnSO_4$ and Zn amino acid chelate (ZnAAC) control diets in Exp. 1, 2, and 3

Each low Zn mean represents 40 pigs in Exp. 1, 32 pigs in Exp. 2, and 16 pigs in Exp. 3. Each control mean represents four pigs in Exp. 1 and eight pigs in Exp. 2

*Low Zn mean differs from $ZnSO_4$ and ZnAAC control means ($P < .01$)

Table 2. Serum chemical profile of pigs fed the low Zn depletion diet for a 32-d period in Exp. 1

Item	Low Zn[a]	SEM	Normal[b]
	----------Minerals----------		
Ca, mg/dL	9.7	.24	9.6
Mg, mg/dL	3.5	.35	2.0
P, mg/dL	14.7	.82	7.3
Na, mEq/dL	141	2.8	145
K, mEq/dL	6.4	.55	6.6
Cl, mEq/dL	95	2.8	102
Anion gap, mEq/dL	26	.55	23
	------Protein and Enzymes------		
Protein, g/dL	7.6	.34	6.1
Albumin, g/dL	3.4	.17	3.5
Urea N, mg/dL	10	1.6	13
AST (SGOT), IU/L	51	9.5	66
GGT, IU/L	49	7.7	47
	----------Other----------		
Glucose, mg/dL	35	7.4	117
CO_2, mEq/L	27	1.1	28
Total bilirubin, mg/dL	.2	.02	.1

[a]Each low Zn mean represents five pigs
[b]Serum chemical profile of genetically similar pigs with ad libitum access to a 19% CP corn soybean meal diet (Schell, 1991)

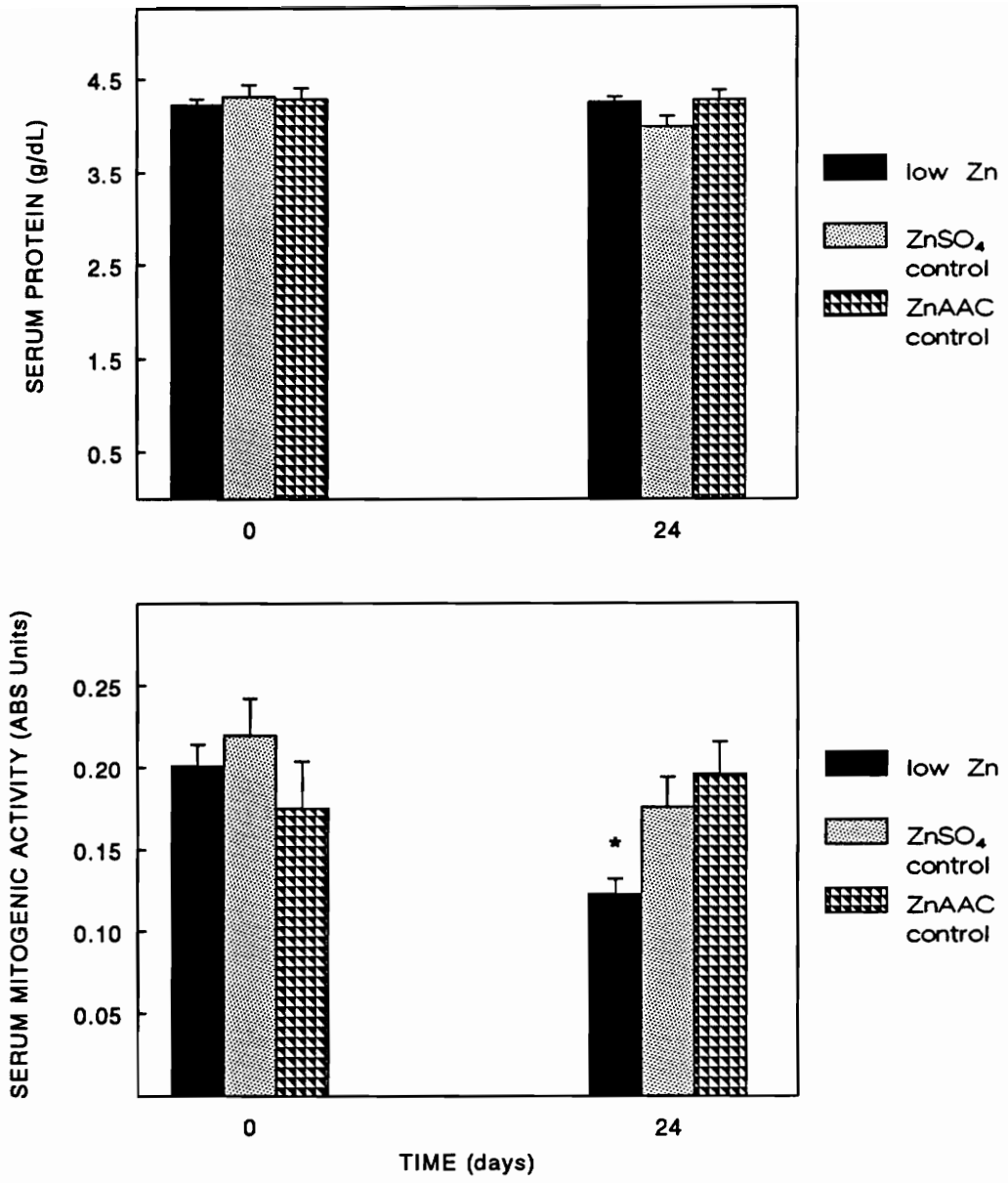

Figure 5. Total serum protein concentration and total serum mitogenic activity of pigs fed the low Zn depletion diet (N=32) and of pigs fed the $ZnSO_4$ and Zn amino acid chelate (ZnAAC) control diets (N=8) before and after Zn depletion in Exp. 2
***Low Zn mean differs from $ZnSO_4$ and ZnAAC control means ($P < .05$)**

Table 3. Performance of pigs fed the low Zn depletion diet and of pigs fed the $ZnSO_4$ and Zn amino acid chelate (ZnAAC) control diets during Exp. 1[a]

Item	Low Zn[b]	Control[b]		SEM
		$ZnSO_4$	ZnAAC	
Days 1 - 7				
ADG, kg	.13	.12	.15	.04
ADFI, kg	.33	.27	.31	.02
Gain:feed	.42	.43	.49	.07
Days 8 - 32				
ADG, kg	.16	.33	.29	.02[c]
ADFI, kg	.51	.53	.52	.02
Gain:feed	.30	.62	.57	.04[c]
Days 1 - 32				
ADG, kg	.15	.28	.26	.02[c]
ADFI, kg	.47	.47	.47	.02
Gain:feed	.32	.59	.56	.03[c]

[a]Starting BW of the low Zn, $ZnSO_4$ and ZnAAC control groups were, respectively, 7.3 $\pm$ .1, 6.8 $\pm$ .4 and 6.8 $\pm$ .4 kg
[b]Each low Zn mean represents ten pens and each control mean one pen (four pigs per pen)
[c]Low Zn mean differs from $ZnSO_4$ and ZnAAC control means ($P < .01$)

Table 4. Performance of pigs fed the low Zn depletion diet and of pigs fed the $ZnSO_4$ and Zn amino acid chelate (ZnAAC) control diets during Exp. 2 and 3[a]

Item	Low Zn[b]	Control[b]		SEM
		$ZnSO_4$	ZnAAC	
Exp. 2, D 1 - 7				
ADG, kg	.16	.17	.17	.04
ADFI, kg	.51	.50	.51	.02
Gain:feed	.30	.33	.34	.07
D 8 - 24				
ADG, kg	.14	.22	.22	.02[c]
ADFI, kg	.45	.49	.48	.01[d]
Gain:feed	.31	.45	.46	.04[d]
D 1 - 24				
ADG, kg	.14	.20	.21	.01[c]
ADFI, kg	.47	.49	.49	.01
Gain:feed	.30	.42	.43	.02[c]
Exp. 3, D 1 - 24				
ADG, kg	.15			.02
ADFI, kg	.42			.04
Gain:feed	.40			.02

[a]Starting BW of the low Zn, $ZnSO_4$ and ZnAAC control groups were, respectively 6.1 $\pm$ .1, 6.4 $\pm$ .2, and 6.2 $\pm$ .2 kg in Exp. 2, and 6.9 $\pm$ .5 kg for the low Zn group in Exp. 3

[b]Each low Zn mean represents ten pens in Exp. 2 and four in Exp. 3, and each control mean two pens (four pigs per pen)

[c,d]Low Zn mean differs from $ZnSO_4$ and ZnAAC control means, respectively, ($P < .01$) and ($P < .05$)

(GF)-ratio. Average daily gain and GF-ratio appeared to be higher for the control pigs in Exp. 1 than in Exp. 2, but performance was not affected by the Zn source.

Serum Growth Factor Activity, Pituitary RNA, and GH mRNA. At the end of Zn depletion in Exp. 2, serum mitogenic activity of Zn depleted pigs was 34 % lower ($P < .05$) compared with the mean of the $ZnSO_4$ and ZnAAC control groups (Figure 5). The low growth factor activity was associated with a low ($P < .01$) total RNA content in the pituitary (Figure 6). Moreover, the GH mRNA fraction of the pituitary RNA tended to be smaller ($P < .10$) for the Zn depleted pigs compared with the $ZnSO_4$ and ZnAAC control groups. Serum growth factor activities and the RNA and GH mRNA content in the pituitary was similar for the $ZnSO_4$ and ZnAAC controls.

Zinc, Cu, and Fe Concentrations in Organ Tissues. Liver and kidney weights of the low Zn group, expressed as a % of BW, were similar to those of the ZnAAC and $ZnSO_4$ control pigs (Table 5). Conversely, relative pancreas weight of the low Zn pigs were lower ($P < .05$) compared with the control groups. Tissue weights of the $ZnSO_4$ and ZnAAC control pigs were similar.

In Exp. 2, liver, kidney, brain, and pancreas Zn concentrations of Zn depleted pigs were lower ($P < .001$) compared with pigs fed the $ZnSO_4$ and ZnAAC control diets (Table 6). Zinc depletion of tissues was most profound in the liver (39 %), followed by the pancreas (35 %), kidney (18 %) and brain (10 %). Tissue Zn concentrations of Zn depleted pigs were clearly lowest in Exp. 1, and appeared to be highest in Exp. 3.

Zinc depletion of organ tissues was only associated with a lower ($P < .001$) Cu level in the kidney (Table 6). Liver, pancreas, and brain concentrations of Cu

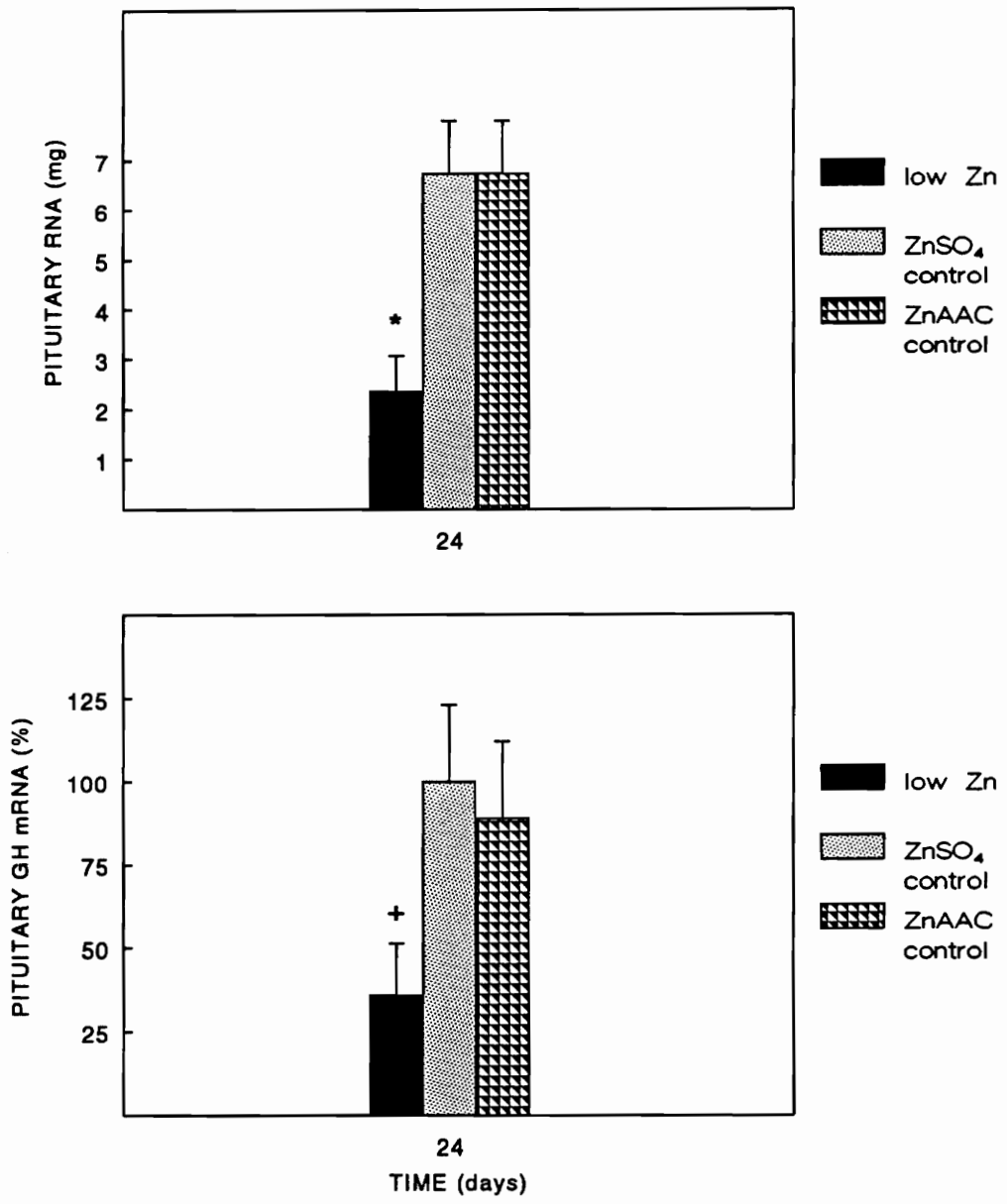

Figure 6. Total pituitary RNA and total pituitary growth hormone (GH) mRNA of pigs fed the low Zn depletion diet (N=8) and of pigs fed the $ZnSO_4$ and Zn amino acid chelate (ZnAAC) control diets (N=4) after 24 d of Zn depletion in Exp. 2
Pituitary GH mRNA is shown relatively to GH mRNA levels of $ZnSO_4$ controls (=100 %)
*Low Zn mean is different from $ZnSO_4$ and ZnAAC control means ($P < .05$)
+Low Zn mean is different from $ZnSO_4$ and ZnAAC control means ($P < .10$)

Table 5. Wet weights, expressed as a % of BW, of organ tissues from pigs fed the low Zn depletion diet and from pigs fed the $ZnSO_4$ and Zn amino acid chelate (ZnAAC) control diets at the end of Zn depletion in Exp. 1, 2 and 3[a]

	Exp. 1	Exp. 2				Exp. 3
			Control[b]			
Tissue	Low Zn[b]	Low Zn[b]	$ZnSO_4$	ZnAAC	SEM	Low Zn[b]
Liver	2.9 ± .14	2.3	2.5	2.1	.23	2.5 ± .22
Kidney	.27 ± .02	.24	.23	.23	.01	.22 ± .02
Pancreas		.11	.14	.14	.01[c]	
Brain		.54	.46	.48	.04[d]	
Pituitary						
($X\ 10^{-4}$)		8.2	7.8	8.1	.6	

[a]Pigs were depleted of Zn for a 32-d period in Exp. 1 and for a 24-d period in Exp. 2 and 3

[b]Each low Zn mean represents six pigs in Exp. 1, eight pigs in Exp. 2, and four pigs in Exp. 3. Each control mean represents four pigs

[c,d]Low Zn mean differs from $ZnSO_4$ and ZnAAC control means, respectively, ($P < .05$) and ($P < .10$)

Table 6. Zinc, Cu, and Fe concentrations (ppm), expressed on a DM basis, of organ tissues from pigs fed the low Zn depletion diet and from pigs fed the $ZnSO_4$ and Zn amino acid chelate (ZnAAC) control diets at the end of Zn depletion in Exp. 1, 2 and 3[a]

	Exp. 1	Exp. 2				Exp. 3
			Control[b]			
Tissue	Low Zn[b]	Low Zn[b]	$ZnSO_4$	ZnAAC	SEM	Low Zn[b]
			Zinc			
Liver	70 ± 2.2	147	264	218	18[c]	161 ± 12
Kidney	87 ± 1.1	132	163	158	4.8[c]	149 ± 2.9
Pancreas		127	194	195	9.5[c]	
Brain		85	95	94	1.0[c]	
			Copper			
Liver	82 ± 12	97	112	98	12	148 ± 29
Kidney	17 ± .8	26	65	56	6.0[c]	34 ± 3.8
Pancreas		7.7	8.4	8.4	.50	
Brain		27	28	27	1.0	
			Iron			
Liver	340 ± 81	377	211	537	90[d]	455 ± 147
Kidney	278 ± 19	317	307	319	30	173 ± 20
Pancreas		101	95	121	11	
Brain		87	84	87	2.3	

[a]Pigs were depleted of Zn for a 32-d period in Exp. 1 and for a 24-d period in Exp. 2 and 3

[b]Each low Zn mean represents six pigs in Exp. 1, eight pigs in Exp. 2, and four pigs in Exp. 3. Each control mean represents four pigs

[c]Low Zn mean differs from $ZnSO_4$ and ZnAAC control means ($P < .01$)

[d]$ZnSO_4$ control mean differs from ZnAAC control mean ($P < .05$)

and Fe, and kidney Fe content were similar for the low Zn and both $ZnSO_4$ and ZnAAC groups.

Zinc, Cu, and Fe Concentrations in Gastrointestinal Tissues. Zinc levels were lower ($P < .01$) in the proximal and medial segments of the small intestine of Zn depleted pigs compared with the $ZnSO_4$ and ZnAAC control pigs (Table 7). Distal intestinal Zn levels were only numerically lower for the Zn depleted pigs. At the end of Zn depletion, intestinal Zn levels of the Zn depleted pigs were reduced by 10 % compared with the $ZnSO_4$ and ZnAAC control pigs.

Intestinal Zn concentrations of the $ZnSO_4$ control group were higher ($P < .05$) in the medial, but not in the proximal and distal segments of the small intestine compared with the ZnAAC controls. Both mucosal and whole intestinal Zn levels were lowest towards the proximal and highest towards the distal end of the small intestine. In Exp. 3, the Zn content of the stomach of Zn depleted pigs was similar to the previously measured intestinal Zn concentrations of Zn depleted pigs in Exp. 2. Pigs in both experiments were subjected to a 24 d Zn depletion period.

Zinc depletion of the small intestine was associated with a high Cu content ($P < .01$) in the proximal segment. The low ($P < .05$) intestinal Fe levels of the low Zn group found in the proximal and medial segments were not clearly associated with Zn depletion of the small intestine. Differences between the low Zn and $ZnSO_4$ and ZnAAC groups resulted primarily from the high ($P < .01$) intestinal Fe concentrations in the small intestine of ZnAAC compared with $ZnSO_4$ control pigs. Intestinal Cu concentrations were similar for the $ZnSO_4$ and ZnAAC control groups.

Intestinal mucosal Cu levels of Zn depleted pigs in Exp. 1 were, like Zn, lower than intestinal Cu levels in Exp. 2. The intestinal mucosal (Exp. 1), however,

Table 7. Zinc, Cu, and Fe concentrations (ppm), expressed on a DM basis, of gastrointestinal tissues from pigs fed the low Zn depletion diet and from pigs fed the $ZnSO_4$ and Zn amino acid chelate (ZnAAC) control diets at the end of Zn depletion in Exp. 1, 2 and 3[a]

	Exp. 1	Exp. 2				Exp. 3
			Control[c]			
Tissue[b]	Low Zn[c]	Low Zn[c]	$ZnSO_4$	ZnAAC	SEM	Low Zn[c]
	----------Zinc----------					
Proximal	78 ± 2.2	119	134	139	4.7[d]	
Medial	88 ± 2.7	125	144	136	2.1[de]	
Distal	91 ± 1.5	147	159	158	9.5	
Stomach						132 ± 1.3
	----------Copper----------					
Proximal	15 ± .8	23	17	15	1.5	
Medial	14 ± .8	19	18	15	2.4	
Distal	11 ± .2	11	12	13	.7	
Stomach						14 ± 1.0
	----------Iron----------					
Proximal	213 ± 31	125	151	223	13.5[df]	
Medial	114 ± 7	82	91	100	5.0[g]	
Distal	127 ± 9	87	89	97	6.9	
Stomach						82 ± 7.3

[a]Zinc depletion period was 32 d in Exp. 1 and 24 d in Exp. 2 and 3
[b]Small intestine was divided in three segments in Exp. 1 and 2 (see text). Intestinal mucosa was collected in Exp. 1 and whole intestine in Exp. 3
[c]Each low Zn mean represents six pigs in Exp. 1, eight pigs in Exp. 2, and four pigs in Exp. 3. Each control mean represents four pigs
[d,g]Low Zn mean differs from control means, respectively, ($P < .01$) and ($P < .05$)
[e,f]$ZnSO_4$ mean differs from ZnAAC mean, respectively, ($P < .05$) and ($P < .01$)

did contain more Fe than the whole intestine (Exp. 2). In both mucosal and whole intestine, Cu and Fe levels were highest towards the proximal and lowest towards the distal end of the small intestine. In Exp. 3, Cu and Fe contents in the stomach of Zn depleted pigs were similar to previously found Cu and Fe concentrations in the small intestine of the low Zn group in Exp. 2.

Discussion

An isolated soy protein, semipurified diet containing 17 ppm of Zn was effectively used to deplete body fluid and tissue Zn pools of weanling pigs within a 3 to 4 wk period. Pathogenesis of parakeratosis in all Zn depleted pigs evolved as previously described by Blood et al. (1979) and Scott (1988).

The severity of Zn depletion was not the same in the three experiments as indicated by the serum ALP-activity and Zn concentrations in liver and kidney. Clearly, pigs fed the low Zn diet in Exp. 1 were most severely depleted of Zn because of the 32-d Zn depletion period. Although pigs were subjected to a 24-d Zn depletion period in both Exp. 2 and 3, pigs in Exp. 3 were less deficient. This may have been due to the higher initial BW and age of the pigs in Exp. 3. Even though the whole body Zn concentration is fairly constant between weaning and marketing (Spray and Widdowson, 1950), the total amount of body Zn, including body Zn reserves, increases with age.

Growth Retardation during Experimental Zn Deficiency. The growth retardation of Zn deficient pigs cannot fully be accounted for by depressions in feed intake as shown by studies, including this one, that used pair-fed controls (Miller et al., 1969; Dørup and Clausen, 1991). It should be pointed out, however, that pair

feeding does not guarantee that the Zn depleted and control groups utilize equal amounts of nutrients. Nutrient utilization of the Zn depleted pigs may have been depressed by a high incidence of moderate to severe diarrhea of the Zn depleted pigs. A high incidence of diarrhea was observed earlier in Zn deficient pigs (Stevenson and Earle, 1956) and has been correlated with poor growth rates and low nutrient intake in pigs fed diets adequate in Zn (Swinkels et al., 1988).

Our findings suggest that the depressions in total serum growth factor activity observed in the Zn depleted pigs resulted from a severely reduced synthesis of pituitary RNA including an even more severe reduction in GH mRNA synthesis. Although Cossack (1986) and Dørup et al. (1991) measured reductions in blood IGF-levels during Zn deficiency in rats, a depression in serum GH levels was not observed (Dørup et al., 1991). This apparent discrepancy which was not found in our study, may have been due to the insensitivity of the method used by Dørup et al. (1991) to measure serum GH levels. The results in this study suggest that growth retardation in Zn deficient animals may be caused by (1) a reduced feed intake, (2) a decreased nutrient availability, (3) a lack of pituitary GH gene expression. A low level of GH synthesis may have depressed the total serum growth factor activity (Mathews et al., 1986; Roberts et al., 1986) which, in turn, decreased protein accretion.

Zinc Depletion in Body Fluids. The more rapid decrease in serum Zn concentrations compared to serum ALP-activity of pigs fed the low Zn diet (Figure 4) was shown earlier in baby-pigs (Miller et al., 1969). Apparently, the portion of Zn in serum which is not a part of biologically active compounds is used to counteract early onsets of Zn deficiency.

The decrease in serum ALP-activity of control pigs in Exp. 2, observed towards the end of Zn depletion (Figure 4), may be due to a low dietary intake of Zn. Conversely, control pigs in Exp. 1 maintained their serum ALP-activity while overall ADFI during Zn depletion was not different between control pigs used in the two experiments (Tables 3 and 4). Control pigs of Exp. 1, however, were more efficient (high GF-ratio) in utilizing nutrients, including Zn, than the control pigs in Exp. 2. Furthermore, age and weight of the pigs at the start of the experiments also may have contributed to the differences in serum ALP-activity between experiments.

The increase in total serum protein concentration and the associated decreases in serum urea-N and glucose contents of the depleted pigs in Exp. 1 (Table 2) was probably due to the low feed intake which is typical in Zn deficient animals. In fasted growing pigs, Kornegay et al. (1964) found similar values for these serum variables although serum glucose concentrations were less depressed. It should be pointed out that the feed intake of depleted pigs in Exp. 1 was extremely low towards the end of Zn depletion, and that blood samples were not taken immediately following a meal.

Zinc Depletion in Body Tissues. Body tissues of animals fed low dietary levels of Zn are not depleted uniformly (Jackson, 1989). In our study, Zn depletion was less severe in brain, small intestinal and kidney tissues than in the pancreas and liver. Bone also is severely depleted of Zn in Zn deficient animals (Giugliano and Millward, 1984; Hill et al., 1986). Muscle, spleen, thymus and testis, however, maintain their Zn levels during Zn deficiency (Canton and Cremin, 1990; Crofton et al., 1983; Giugliano and Millward, 1984). It may be that the most severely Zn depleted tissues contain a relatively larger portion of the body Zn reserve.

Systemic Interactions between Zn, Cu, and Fe during Zn Depletion. Zinc depletion affected kidney Cu levels, but not liver and pancreas Cu contents in this study (Table 6). Moreover, bone Cu content of pigs depleted with a corn soybean meal diet with no supplemental Zn also was not associated with Zn depletion (Hill et al., 1986). To date, little is known regarding the mechanisms underlying systemic mineral interactions. It is not possible to infer any feasible mode of action from our data. However, we may suggest that mechanisms underlying systemic interactions between Cu and Zn are not general in nature across body tissues.

The high Cu levels in the proximal segment of the small intestine (Table 7) may have resulted from a competition between Zn and Cu for intestinal metallothionein (Cousins, 1985). Dietary Cu levels used in this study were low (16 ppm). Therefore, Cu, the mineral which is preferred by metallothionein (Dunn et al., 1987), may only have bound metallothionein in pigs fed the low Zn diet at intestinal site(s) where Zn compared with Cu absorption was low. Since metallothionein levels are reduced in Zn deficient animals (Hoadley et al., 1988), Zn was likely bound to metallothionein at intestinal site(s) where Zn compared with Cu absorption was high. In the control groups, absorption rates of Zn apparently were high throughout the small intestine. Therefore, Cu was probably not able to compete with Zn for metallothionein binding sites in pigs fed the control diets.

The low Fe concentration in the small intestine of pigs fed the low Zn diet was probably due to a depressed availability of nutrients. As discussed previously, nutrient availability may have been reduced in Zn depleted pigs due to a high incidence of diarrhea.

Implications

Feeding an isolated soy protein, semipurified diet (17 ppm Zn) depresses growth rate and feed intake, and depletes body fluid and tissue stores of Zn in pigs. The depression in growth rate may be due to reduced activity of growth factors primarily resulting from a general shut down of pituitary RNA synthesis, including GH mRNA. In addition to serum Zn concentrations and serum ALP-activity, serum growth factor activity may be useful as a diagnostic indicator for assessing Zn status in young growing humans and animals.

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CHAPTER IV

Comparison of an Inorganic and Organic Zinc Source. I. Site and Rate of Zn Absorption and Zn Retention from an Amino Acid Chelate in Zn Depleted Pigs

Abstract

In Exp. 1, Zn depleted pigs were fed an isolated soy protein, semipurified diet supplemented with 15 and 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) to determine the site and rate of apparent Zn, Cu, and Fe absorption in Zn depleted pigs. The indirect method with chromic oxide was used. In a follow-up experiment (Exp. 3), apparent Zn, Cu, and Fe absorption was measured only within the stomach and colon, and apparent Zn, Cu, and Fe absorption, apparent DM digestibility, and Zn, Cu, and Fe retention were determined during a 7-d balance study. In Exp. 1, apparent Zn absorption coefficients of depleted pigs fed the 15 and 45 ppm ZnAAC diets were higher ($P < .01$) than those of pigs fed the respective $ZnSO_4$ diets. The major difference seemed to occur within the stomach, where apparent absorption coefficients were negative for the $ZnSO_4$ and positive for the ZnAAC group. However, in Exp. 3, apparent Zn absorption coefficients within the stomach of both $ZnSO_4$ and ZnAAC fed pigs were low and similar for both the $ZnSO_4$ and ZnAAC fed pigs. In both experiments, apparent Cu and Fe absorption, and apparent DM digestibility were not affected by Zn source, except for ZnAAC fed pigs in Exp. 3 where apparent Fe absorption within the colon was higher ($P < .05$) compared with the $ZnSO_4$ group.

In Exp. 1, Zn and Cu absorption occurred primarily within jejunal and ileal segments of the small intestine. Additionally, Cu absorption also occurred within in the stomach. Iron was mostly absorbed within duodenal and jejunal segments of the small intestine. In the balance of Exp. 3, a higher ($P < .01$) disappearance rate of Zn, Cu, Fe and DM, and a lower ($P < .01$) fecal moisture content (41.4 vs 52.4 %), were found for pigs fed ZnAAC compared with $ZnSO_4$. Moreover, Cr recovery was lower for pigs fed ZnAAC compared with $ZnSO_4$ (70.2 vs 87.1 %) suggesting a greater ($P < .01$) disappearance of Cr for the ZnAAC compared with $ZnSO_4$ fed pigs. In summary, colonic apparent Zn absorption coefficients, calculated by the indicator method, were improved by using ZnAAC instead of $ZnSO_4$ in Exp. 1, but not in Exp. 3 unless corrected for Cr recovery. Higher disappearance rates of Zn, Cu, Fe, and DM, a lower fecal moisture content, and a lower Cr recovery measured in pigs fed ZnAAC compared with $ZnSO_4$ clearly indicates that intestinal luminal conditions were affected by the ZnAAC.

(Key words: mineral absorption, pigs, zinc depleted, zinc chelate, zinc sulfate, chromic oxide)

Introduction

Absorption of Zn is a complex and, although extensively studied, not well-understood process (Cousins, 1989). The initial phase of Zn absorption, the uptake of Zn from the intestinal lumen, involves both carrier-mediated (Davies, 1980) and non-mediated (Tacnet et al., 1990) components. After cellular uptake, Zn is proposed to be bound to one of several low molecular weight proteins present in the

intestinal mucosa, viz. metallothionein, which may function in Zn homeostasis (Starcher et al., 1980; Hempe and Cousins, 1992).

Zinc in the gastrointestinal (GI) lumen exists mostly bound to chemical ligands of dietary or secretory origin, which may inhibit or facilitate Zn absorption (Kratzer and Vohra, 1986; Lönnerdal, 1991). Using perfused rat jejunal and ileal segments, Wapnir and Stiel (1986) observed an increase of apparent Zn absorption after adding tryptophan, histidine or proline to the perfusate containing .153 mM $ZnSO_4$. Complexing methionine with Zn, however, decreased Zn absorption by ligated rat duodenal loops (Hempe and Cousins, 1989).

In rats, duodenal (Davies, 1980) and ileal segments (Antonson et al., 1979) of the small intestine have been designated as primary sites of Zn uptake. Chemical ligands, however, may play a role in determining the site of Zn absorption. Uptake of Zn from an isolated duodenal segment of rat intestine was reduced after addition of a high level of histidine to the perfusate, whereas, Zn uptake was enhanced in jejunal and ileal segments (Schwarz and Kirchgessner, 1975). Inclusion of high levels of cellulose in swine diets, reduced rates of apparent Zn absorption measured in the large intestine which were partially offset by enhanced absorption of Zn at site(s) anterior to the terminal ileum (Partridge, 1978).

The objective of the present study was to determine the site and rate of apparent Zn absorption and to compare Zn retention from a Zn amino acid chelate and $ZnSO_4$ in Zn depleted pigs fed a semipurified diet.

Experimental Procedures

Two experiments were conducted using a total of 42 Zn depleted pigs. These experiments were part of a study to compare the availability of Zn from a Zn amino acid chelate (Albion Laboratories, Cadco Inc., Des Moines, IA) and $ZnSO_4.7H_2O$. Dietary treatments and experimental procedures were described in detail in Chapters III and V and will only be briefly summarized. To be consistent with Chapters III and V, the experiments will be referred to as Exp. 1 and 3.

Animals, Diets, and Housing. In Exp. 1, 30 pigs were used that had been depleted of Zn for 32 d by feeding a low Zn depletion diet that provided 17 ppm Zn (Table 1). In Exp. 3, 12 pigs were used that had been Zn depleted for 24 d using the same low Zn diet as in Exp. 1. The low Zn diet also was used as basal for the Zn repletion diets.

In Exp. 1, depleted pigs were repleted up to 24 d using the low Zn diet supplemented with 5, 15, or 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC). Six pigs (one per treatment) were sacrificed on d 0, 3, 6, 12 and 24 (Figure 1).

In Exp. 3, depleted pigs were repleted for 15 d using the low Zn diet supplemented with 45 ppm Zn, either as $ZnSO_4$ or as ZnAAC. After an adjustment period of 7 d, urine and feces were collected for a 7-d period (Figure 1). At the end of the 15-d Zn repletion period all pigs (six per treatment) were sacrificed.

In both experiments, pigs were fed twice daily starting at 0600 and 1800. The daily feeding level was 2.5 times maintenance (maintenance = 500 kJ ME per kg $BW^{\cdot 75}$). Pigs were allowed access to the feed for 2 h, after which left over feed was

Table 1. Composition of the semipurified diet

Ingredient	(g per kg diet)
Corn starch[a]	336.6
Dextrose[b]	300
Isolated soy protein[c]	200
Corn oil	60
Cellulose[d]	30
Dynafos[e]	29
Mineral mix[f]	16.6
Dyna-K[e]	14.4
$CaCO_3$	5.8
Vitamin mix[g]	4
Dynamate[e]	3.6

[a]Cargill Inc., Minneapolis, MN
[b]Cerelose Dextrose 2001, Corn Products, Summit-Argo, IL
[c]PP500E, Protein Technologies International, St. Louis, MO
[d]Purified powder cellulose BH200, International Filler Co., North Tonawanda, NY
[e]Dynafos contained (minimum) 20% Ca, 18.5% P, and (maximum) .185% F; Dynamate contained (minimum) 22% S, 18% K, and 11% Mg; Dyna-K contained (minimum) 96.5% KCl and 50% K. Pitman-Moore Inc., Mundelein, IL
[f]Provided the following sources of minerals in g per kg of diet: $FeSO_4.7H_2O$, .25; $CuSO_4.5H_2O$, .024; $MnSO_4.H_2O$, .012; KIO_3, .00024; Na_2SeO_3, .00066; dextrose, 15.3
[g]Provided the following amounts per kg of diet: vitamin A, 4,950 IU; vitamin D_3, 660 IU; vitamin E, 33 IU; vitamin K, 6 mg; riboflavin, 3.3 mg; niacin, 35.2 mg; pantothenic acid, 19.8 mg; vitamin B_{12}, .022 mg; choline, 1,184 mg; thiamin, 2.6 mg; vitamin B_6, 3 mg; biotin, .4 mg; folacin, 2mg; D,L-methionine, 1,200 mg

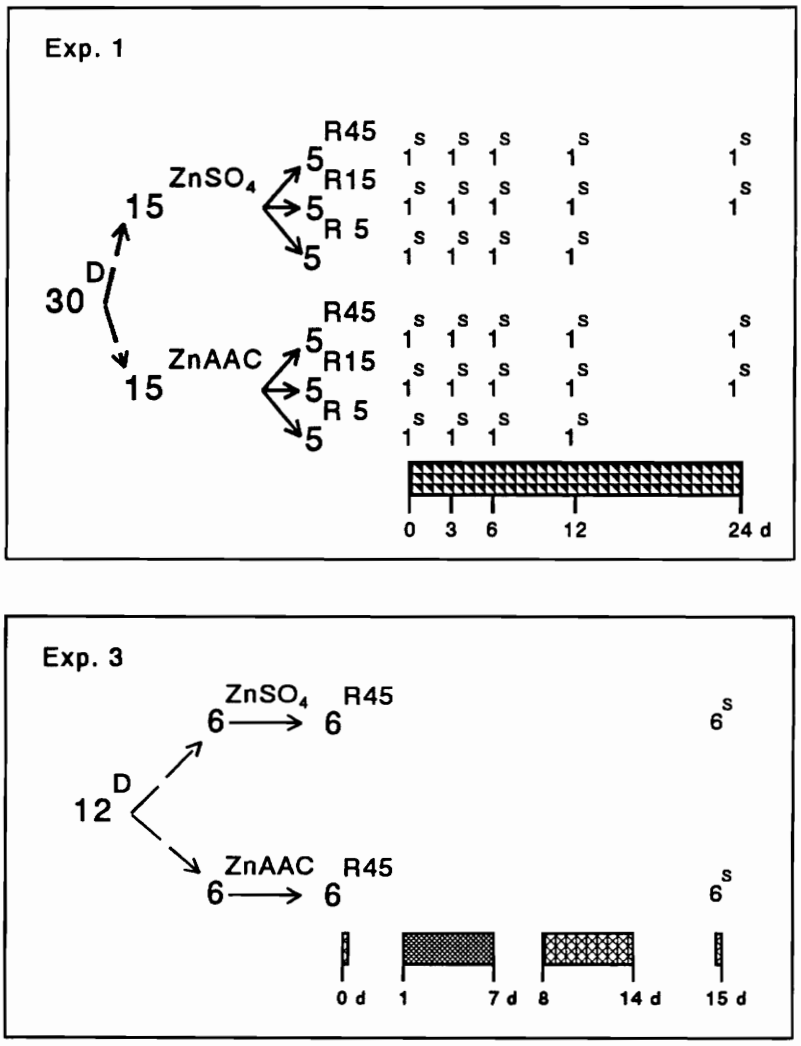

Figure 1. Schematic overview of experimental design and measurements of Exp. 1 and 3
Legend: D = Zn depleted, ZnAAC = Zn amino acid chelate, R# = repleted + level of supplemental Zn, S = sacrificed

Zn repletion period adaptation period collection period

collected and weighed. Deionized water was provided continuously using nipple waterers. Other experimental details are presented in Chapter V.

Experiment Conduct, Sample Collection, and Sample Analyses. In Exp. 1, an inert solid phase marker, .25% chromic oxide, was included in the diet at least one wk prior to digesta collection. The digesta of the GI tract was collected following a procedure that was modified from Asche et al. (1989). Briefly, pigs were sacrificed by intravenous injection of a lethal dose (.25 mL per kg BW) of sodium pentobarbital (Anthony Products, Arcadia, CA). Additionally, each pig was injected intraperitoneally with 3 mL of sodium pentobarbital to reduce mucosal shedding and to minimize digesta movement resulting from small intestine contractions. Pigs were exsanguinated prior to digesta collection to minimize blood contamination of GI contents.

The digesta were collected exactly 2.5 h after offering the meal to the pig. This was achieved by using a staggered feeding regimen in which pigs were fed at 35-min intervals throughout the last week of Zn depletion and the entire 24-d Zn repletion period. On the first day of digesta collection, the order of sacrifice for the pigs of the six different treatments was randomly determined. Each subsequent collection day, the order of sacrifice for each treatment moved up by one, except the treatment previously sacrificed last became first in order.

After exsanguination, the abdominal cavity was opened using blunt scissors to avoid cutting the GI tract. The GI tract was ligated into the following seven segments using plastic ties (Part #: 95476, Consolidated Plastic Co., Twinsburg, OH): (1) stomach, (2) proximal small intestine (one-third distal of pylorus), (3) medial small intestine (one-third distal of pylorus to one-third proximal of ileo-cecal valve), (4) distal small intestine (one-third proximal of ileo-cecal valve), (5) cecum,

(6) proximal colon (one-half distal of ileo-cecal valve), and (7) distal colon (one-half proximal of anus). Digesta were gently expressed from each segment of the GI tract and collected into acid washed plastic containers. Each of the GI segments was gently rinsed with physiological saline (stomach, 50 mL; small intestine and large intestine segments, 10 mL) to ensure complete collection of the digesta. The collected digesta were weighed and stored on ice (4°C) until further processed.

In Exp. 3, .25% chromic oxide was included in the diet at the beginning of the 15-d Zn repletion period. After 7 d of adaptation, feces were collected twice daily in ziploc bags and stored in plastic containers, and urine was collected in plastic containers during a 7-d period. At the end of the 7-d collection period, feces and urine weights were recorded. When pigs were sacrificed, the stomach and colon were ligated and excised. Stomach and colon digesta were collected as described for Exp. 1.

In Exp. 1, digesta were sampled and dried in a forced vent oven for 48 h at 105°C. In Exp. 3, feces were dried in a forced vent oven for 24 h at 70°C. After drying, both digesta and feces were weighed. Digesta were ground using a mortar and pestle, and feces were ground to pass a 1 mm screen using a cyclone type sample mill (Cyclotec Sample Mill, Tecator, AB).

Feed, feces and GI digesta of each segment were analyzed for Cr, Zn, Cu and Fe using flame atomic absorption spectrophotometry (Perkin Elmer 5100, Cornwalk, CT) following wet digestion with HNO_3 and $HClO_4$ (AOAC, 1990). Urine Zn, Cu, Fe and Cr concentrations were determined using flame atomic absorption spectrophotometry. Dry matter content was determined on feed, digesta and feces by drying for 24 h in a forced vent oven (105°C).

Apparent absorption coefficients for Zn, Cu and Fe (%) were determined indirectly using the following equation:

$$100 - 100 * \frac{(\%\ \text{Cr in feed} * \%\ \text{nutrient in feces})}{(\%\ \text{Cr in feces} * \%\ \text{nutrient in feed})}.$$

Statistical Procedures. Apparent absorption coefficients of Exp. 1 were analyzed by the GLM procedure of SAS (1988) using split plot analysis (Steel and Torrie, 1980). The whole units of the model were Zn source ($ZnSO_4$ and ZnAAC), Zn level (15 and 45 ppm Zn) and day (d 0, 3, 6, 12 and 24). The GI segment was the subunit of the model. The whole units and their two-way interactions were tested against Zn source x Zn level x day, which defines the pig as the experimental unit. The subunit and its two- and three-way interactions with, respectively, Zn source, Zn level and day were tested against the residual error term. Since the three-way interaction GI segment x Zn level x day was non-significant for all variables, it was included in the error term. Orthogonal contrasts were used to test for linear, quadratic and cubic apparent absorption patterns measured from within the stomach to within the distal colon. Furthermore, orthogonal contrasts were used to test the depleted pigs sacrificed on d 0 against the depleted pigs sacrificed during Zn repletion, and to test depleted pigs sacrificed on d 3 and 6 vs d 12 and 24.

Mineral and DM balance data, and mineral apparent absorption coefficients of Exp. 3 were tested by the GLM procedure of SAS (1988). Zinc source was the only main effect of the model and the pig functioned as the experimental unit.

Results

General. Depleted pigs which were fed the low Zn depletion diet supplemented with 5 ppm Zn either as $ZnSO_4$ or ZnAAC did not regain their appetite and ability to grow within 12 d. Consequently, amounts of digesta at the days of digesta collection were extremely small which made accurate mineral analyses impossible. Hence, apparent absorption coefficients of these pigs were not included. Serum Zn, serum ALP-activity, and performance data of both 5 ppm Zn groups were presented in Chapter V.

The low Zn diet was analyzed and found to contain 17 ppm Zn, 16 ppm Cu and 480 ppm Fe. The $ZnSO_4$ diets contained 26 and 59 ppm Zn and the ZnAAC diets 34 and 65 ppm Zn, respectively, for the 15 and 45 ppm supplemental Zn levels.

Apparent Absorption of Zn. In Exp. 1, large SEM, in particular within the proximal and medial segments of the small intestine, were indicative of considerable variability among apparent absorption coefficients. Nevertheless, the apparent absorption of Zn within the stomach was positive for the ZnAAC group and negative for pigs fed the $ZnSO_4$ diet (Table 2). In both groups, Zn absorption occurred primarily within the medial and distal segments of the small intestine, and was preceded by secretion of Zn into the proximal segment of the small intestine. There appeared to be little absorption of Zn within the three segments of the large intestine. Apparent absorption coefficients of Zn within the GI segments appeared to vary between Zn sources, levels of Zn and among days as indicated by the two- and three-way interactions ($P < .05$) involving the three whole units (Zn source, Zn level, day) and the subunit (GI segment).

Table 2. Apparent absorption coefficients of Zn determined within gastrointestinal (GI) segments of depleted pigs fed the low Zn depletion diet supplemented with 15 and 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) in Exp. 1[a]

	Depleted[b]				
	$ZnSO_4$		ZnAAC		
GI segment[cdefghi]	15	45	15	45	SEM
	----------%				
Stomach	-20.4	-16.1	16.8	14.6	6.6
Small intestine					
Proximal	-24.3	-46.9	-9.2	-19.5	18.7
Medial	5.4	.7	-34.2	29.0	14.1
Distal	25.5	17.6	37.4	39.7	5.8
Large intestine					
Cecum	18.0	18.4	36.3	37.6	4.7
Colon					
Proximal	20.3	15.7	29.6	31.9	4.9
Distal	16.7	17.4	35.2	38.6	6.2

[a]GI tract was divided into seven segments (see text)
[b]Each depleted mean represents five pigs, sacrificed on d 0, 3, 6, 12, and 24 (one pig per d)
[c]Zn source effect ($P < .01$)
[d]Zn source by day interaction ($P < .10$)
[e]Linear, quadratic and cubic increases from stomach to distal colon segment ($P < .05$)
[f]Zn source by GI segment interaction ($P < .05$)
[g]Zn level by GI segment interaction ($P < .01$)
[h]Zn source by Zn level by GI segment interaction ($P < .01$)
[i]Zn source by day by GI segment interaction ($P < .01$)

Conversely to findings in Exp. 1, apparent Zn absorption coefficients within the stomach and colon were similar for pigs fed ZnAAC compared with pigs fed $ZnSO_4$ (Table 6). In Exp. 3, however, the percent recovery of Cr was higher ($P < .01$) for pigs fed $ZnSO_4$ compared with ZnAAC. Consequently, colonic apparent Zn absorption coefficients were, like in Exp. 1, higher for pigs fed ZnAAC compared with $ZnSO_4$, when corrected for Cr recovery.

Apparent Absorption of Cu. In Exp. 1, Cu absorption occurred primarily within the stomach and the medial and distal small intestinal segments of all depleted pigs (Tables 3 and 6). In Exp. 3, Cu absorption was also measured within the stomach of all depleted pigs, but proportionately it contributed less to net Cu absorption. There was negligible absorption of Cu in the large intestine of the depleted pigs fed the 15 and 45 ppm $ZnSO_4$ and ZnAAC diets. Copper apparent absorption coefficients of depleted pigs fed the 15 ppm ZnAAC diet were similar to those observed for the 45 ppm ZnAAC group, except in the medial segment of the small intestine where the Cu absorption coefficient was negative for the 15 ppm ZnAAC group. In general, apparent absorption of Cu was not affected by Zn source or level of Zn.

Apparent Absorption of Fe. In Exp. 1, Fe absorption occurred primarily within the proximal and medial segments of the small intestine (Table 4). However, there was considerable unexplainable variability of coefficients for the proximal segment with values ranging from 28.7 % to a negative 29.2 %. Both stomach and cecum of all depleted pigs were sites at which Fe was secreted as indicated by the negative apparent absorption coefficients. A modest net apparent absorption of Fe was measured for the depleted pigs fed the low Zn diet supplemented with 15 and 45 ppm Zn either as $ZnSO_4$ or ZnAAC. In Exp. 3, like in Exp. 1, Fe was secreted

Table 3. Apparent absorption coefficients of Cu determined within gastrointestinal (GI) segments of depleted pigs fed the low Zn depletion diet supplemented with 15 and 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) in Exp. 1[a]

	Depleted[b]				
	$ZnSO_4$		ZnAAC		
GI segment[cdef]	15	45	15	45	SEM
	----------%				
Stomach	45.5	34.0	36.8	41.8	9.1
Small intestine					
Proximal	36.2	23.1	40.9	30.3	10.4
Medial	49.8	31.2	-1.9	41.2	16.5
Distal	49.8	38.2	50.9	48.7	8.2
Large intestine					
Cecum	48.6	35.7	36.1	41.7	4.0
Colon					
Proximal	46.6	32.5	31.0	39.2	4.4
Distal	51.9	39.1	41.1	48.6	3.4

[a]GI tract was divided into seven segments (see text)
[b]Each depleted mean represents five pigs, sacrificed on d 0, 3, 6, 12, and 24 (one pig per d)
[c]Linear increase from stomach to distal colon segment ($P < .05$)
[d]Zn source by GI segment interaction ($P < .05$)
[e]Zn source by Zn level by GI segment interaction ($P < .05$)
[f]Depleted pigs sacrificed on d 0 are different from depleted pigs sacrificed on d 3, 6, 12, and 24 ($P < .10$)

Table 4. Apparent absorption coefficients of Fe determined within gastrointestinal (GI) segments of depleted pigs fed the low Zn depletion diet supplemented with 15 and 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) in Exp. 1[a]

	Depleted[b]				
	$ZnSO_4$		ZnAAC		
GI segment[cdef]	15	45	15	45	SEM
	----------%----------				
Stomach	-14.9	-24.3	-20.2	-34.6	9.3
Small intestine					
Proximal	28.7	.7	-29.2	9.0	25.6
Medial	43.4	36.1	15.3	35.8	14.2
Distal	44.0	34.2	49.0	48.0	8.6
Large intestine					
Cecum	-9.8	-29.5	-53.9	-10.1	21.0
Colon					
Proximal	12.2	13.7	2.8	12.7	7.3
Distal	19.7	11.5	12.2	15.9	5.0

[a]GI tract was divided into seven segments (see text)
[b]Each depleted mean represents five pigs, sacrificed on d 0, 3, 6, 12, and 24 (one pig per d)
[c]Zn source effect ($P < .05$)
[d]Linear, quadratic and cubic increases from stomach to distal colon segment ($P < .05$)
[e]Zn source by Zn level interaction ($P < .01$)
[f]Zn source by day interaction ($P < .10$)

Table 5. Apparent digestibility coefficients of DM determined within gastrointestinal (GI) segments of depleted pigs fed the low Zn depletion diet supplemented with 15 and 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) in Exp. 1[a]

	Depleted[b]				
	$ZnSO_4$		ZnAAC		
GI segment[cdefgh]	15	45	15	45	SEM
	----------%----------				
Stomach	-1.0	-.7	7.5	5.0	2.7
Small intestine					
Proximal	1.1	36.0	13.7	15.3	14.1
Medial	59.1	70.2	46.9	56.4	5.9
Distal	83.4	83.7	84.5	85.2	1.0
Large intestine					
Cecum	89.4	89.3	88.4	90.9	.8
Colon					
Proximal	90.9	91.2	90.0	91.7	.4
Distal	91.2	91.9	91.1	92.6	.2

[a]GI tract was divided into seven segments (see text)
[b]Each depleted mean represents five pigs, sacrificed on d 0, 3, 6, 12, and 24 (one pig per d)
[c]Zn level effect ($P < .05$)
[d]Linear, quadratic and cubic increases from stomach to distal colon segment ($P < .01$)
[e]Zinc level by GI segment interaction ($P < .05$)
[f]Day by GI segment interaction ($P < .01$)
[g]Zinc source by Zn level by GI segment interaction ($P < .05$)
[h]Depleted pigs sacrificed on d 3 and 6 are different from depleted pigs sacrificed on d 12 and 24 ($P < .10$)

Table 6. Apparent absorption coefficients of Zn, Cu, Fe, and apparent digestibility of DM determined within gastrointestinal (GI) segments, and Cr recovery in depleted pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) after 15 d of Zn repletion in Exp. 3

Item	Depleted[a]		SEM
	$ZnSO_4$	ZnAAC	
	-------Zinc, %-------		
Stomach[b]	3.4	3.1	2.2
Colon[b]	32.7 (41.2)[c]	30.7 (51.4)	1.9
	------Copper, %------		
Stomach	18.5	13.3	2.1
Colon	38.7 (46.1)	35.5 (54.2)	3.3
	--------Iron, %------		
Stomach	-5.2	-20.3	11.5
Colon	11.7 (23.8)	20.0 (43.8)	2.9[d]
	----Dry matter, %----		
Stomach	14.2	11.0	1.8
Colon	93.1 (94.0)	92.8 (94.9)	.2
	----Cr recovery, %---		
	87.1	70.2	1.4[d]

[a]Stomach and colon were excised from the GI tract (see text)
[b]Each depleted mean represents six pigs
[c]Corrected for Cr recovery
[d]Zn source effect ($P < .05$)

into the stomach of both the $ZnSO_4$ and ZnAAC fed pigs, but a higher ($P < .05$) net absorption of Fe was measured within the colon of the ZnAAC fed pigs compared with the $ZnSO_4$ group.

Apparent Digestibility of DM. In Exp. 1, DM digestibility occurred throughout the small intestine of all depleted pigs, but the medial and distal segments of the small intestine were the primary sites (Table 5). A small portion of DM was digested within the stomach of depleted pigs fed the 15 and 45 ppm ZnAAC diets in Exp. 1, and in the stomach of pigs fed both the $ZnSO_4$ and ZnAAC diets in Exp. 3 (Table 6). The large intestine segments of all depleted pigs contributed little to net DM digestion. Dry matter digestibility was not clearly affected by the Zn source, but was higher ($P < .05$) for pigs fed the low Zn diet supplemented with 45 ppm compared with 15 ppm Zn. In Exp. 3, DM digestibility measured within the colon appeared to be slightly higher than DM digestibility measured within the distal colon in Exp. 1.

Apparent Absorption from Balance Data. In Exp. 3, apparent absorption and retention of Zn, Cu, and Fe, and apparent DM digestibility of depleted pigs fed the ZnAAC diet were higher ($P < .01$) compared with pigs fed the $ZnSO_4$ diet (Table 7). The difference in retention of the minerals resulted from apparent absorption differences, because urinary excretion of minerals was negligible and similar for the ZnAAC and $ZnSO_4$ groups.

Discussion

Rate of Apparent Zn, Cu, and Fe Absorption. The apparent absorption coefficients of Zn, Cu and Fe in our study were similar to coefficients obtained

Table 7. Apparent absorption and retention of Zn, Cu, and Fe, and apparent DM digestibility of depleted pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) in Exp. 3

	Depleted[a]		
Balance (7 d)	$ZnSO_4$	ZnAAC	SEM
	--------Zinc--------		
Intake, mg	215	195	13.6
Feces, mg	140	103	8.7[b]
Urine, mg	4.0	4.2	.5
Absorbed, mg	75	92	8.4
Retained, mg	71	88	8.6
Absorption, %	34.6	47.3	2.6[c]
	--------Copper-------		
Intake, mg	30.9	27.8	1.9
Feces, mg	27.4	20.3	1.6[c]
Urine, mg	1.2	.9	.09
Absorbed, mg	3.5	7.6	1.2[b]
Retained, mg	2.3	6.6	1.2[b]
Absorption, %	10.5	27.3	3.8[c]
	---------Iron---------		
Intake, g	1.63	1.48	.10
Feces, g	1.16	.83	.07[c]
Urine, g	.006	.007	.001
Absorbed, g	.47	.65	.05[b]
Retained, g	.46	.64	.05[b]
Absorption, %	28.6	43.9	1.9[c]

Table 7 (continued)

	------Dry matter-----		
Intake, kg	3.42	3.06	.21
Feces, kg	.21	.16	.01[b]
	(52.4)[d]	(41.4)	(2.5)[c]
Digested, kg	3.21	2.90	.20
Digestibility, %	93.7	94.7	.1[c]

[a]Each depleted mean represents six pigs
[b,c]Zn source effect, respectively, ($P < .05$) and ($P < .01$)
[d]Dry matter content of wet feces; feces of the $ZnSO_4$ and ZnAAC groups contained 47.6 % and 58.6 % moisture, respectively

when Zn adequate grower pigs were fed a corn soybean meal diet containing NRC recommended levels of Zn, Cu and Fe (Moore and Kornegay, 1987; Moore et al., 1988; NRC, 1988; Ravindran et al. 1984). Large increases in Zn absorption have been found in rats with a high body need for Zn (Hallmans et al., 1987; Menard and Cousins, 1983). Although depleted pigs in our study were clearly Zn deficient, they were not able to markedly increase their capacity for Zn absorption. Alternatively, it may be that fast growing pigs fed a corn soybean meal diet containing NRC recommended levels of Zn adapt to a high level of Zn absorption to maintain an adequate Zn supply.

Sites of Zn, Cu, and Fe Absorption. In most studies sites of mineral absorption were determined using everted gut sacs, ligated intestinal loops or canulation techniques (Antonson et al., 1979; Davies, 1980; Partridge, 1978; Schwarz and Kirchgessner, 1975; Seal and Heaton, 1983). In our study, the sites of absorption of Zn, Cu and Fe were determined in vivo in the presence of dietary ligands. Furthermore, the entire GI tract was considered. Our findings suggest that both jejunal and ileal segments of the small intestine are the most important sites of Zn absorption (Table 2). Excretion of Zn observed in the proximal segment of the small intestine is probably due to pancreatic secreta, including Zn containing metalloenzymes. Negligible absorption of Zn was measured in the stomach and large intestine of depleted pigs.

Apparent absorption of Cu occurred in the stomach and, like Zn, in the jejunal and ileal segments of the small intestine (Table 3). In chickens, Cu absorption from the proventriculus was suspected by Miller and Jensen (1966) after measuring a net Cu uptake in the gizzard. The relative contribution of the stomach to the net absorption of Cu may be inflated in this study because of the low dietary

levels of Cu (16 ppm). Nevertheless, the stomach should be considered as a site of Cu uptake when studying the mechanisms of Cu absorption.

In contrast to Zn and Cu, large amounts of Fe were excreted into the large intestine. It is not clear, why the high apparent absorption of Fe within the small intestine was followed by even higher Fe excretions into the large intestine. Since Fe balance is believed to be regulated by means of altering the rate of small intestinal Fe absorption (Savin and Cook, 1980), the Fe excretions most likely serve a specific purpose different from removal of excess body Fe. The apparent absorption patterns of Fe observed in this study warrants further investigation into the regulatory mechanisms underlying Fe absorption and metabolism.

Zn Amino Acid Chelate Alters Intestinal Luminal Conditions. The 87 % Cr recovery in feces of pigs fed the $ZnSO_4$ diet was within the range of 83 to 93 % previously found in human subjects (Sharpe and Robinson, 1970; Allen et al., 1979), but the 70 % fecal Cr recovery of depleted pigs fed ZnAAC was low (Table 6). The lack of Cr recovery in pigs fed ZnAAC was associated with a high disappearance rate of Zn, Cu, Fe and DM and with a high moisture content of fecal matter.

An explanation for the discrepancies between the outcome of the indicator and balance data would be that the apparent absorption of Zn, Fe, Cu and Cr as well as the apparent DM digestibility were improved when ZnAAC instead of $ZnSO_4$ was used. Compounds of Cr^{3+}, like the marker chromic oxide used in this study, are the most common and stable oxidation state of Cr (Nicholls, 1975). Since chromic oxide is kinetically inert, i.e., undergoing only slow ligand substitution reactions, it seems unlikely that dissociation of chromic oxide occurred within the gastrointestinal tract. Although absorption of free inorganic, trivalent Cr was measured within the small intestine of rats (Dowling et al., 1992), it is generally

assumed that Cr in the form of chromic oxide is not absorbed. Increased absorption rates of Zn, Cu and Fe and a higher DM digestibility for pigs fed ZnAAC instead of $ZnSO_4$ may have occurred. However, this was not supported by the availability data of these and genetically similar pigs (Chapter V).

An alternative explanation for the incomplete Cr recovery in both $ZnSO_4$ and ZnAAC fed pigs would be that a 7-d adaptation period was insufficient to achieve a Cr equilibrium. Furthermore, the difference in Cr recovery between the two treatments suggests that the luminal residence time of Cr was higher in pigs fed the ZnAAC compared with $ZnSO_4$ diet. The residence time of Cr could possibly be influenced by the gastrointestinal surface area. In a study with miniature pigs, Fleming et al. (1992) found that a lower viscosity of digesta was associated with an increased proliferation of cecal mucosal cells thereby increasing the cecal crypt depth. The moisture content of the feces in our study appears to be indicative for a lower viscosity of digesta from pigs fed the ZnAAC diet compared with the $ZnSO_4$ diet. Therefore, it may be that intestinal mucosal cell proliferation was affected by the ZnAAC in the cecum and perhaps in the small intestine. Longer villi and deeper crypts in the brushborder membrane of small intestinal, epithelial cells would increase the small intestinal surface area necessary for increasing the residence time of Cr. Furthermore, endogenous losses resulting from mucosal sloughing would be reduced. This may explain the higher apparent absorption of minerals and greater apparent DM digestibility found for the pigs fed ZnAAC during the balance period of Exp. 3. Since intestinal morphology was not examined in our study, the arguments remain highly speculative. Further research is warranted to examine a possible influence of ZnAAC on proliferation of intestinal mucosal cells.

Implications

The GI sites with the highest capacity for Zn absorption were, jejunum and ileum for Zn, stomach, jejunum and ileum for Cu, and duodenum and jejunum for Fe. Based on the uncorrected indicator data, rate and site of apparent Zn, Cu, and Fe absorption were not clearly influenced by the Zn source. However, balance data indicated that the disappearance rates of Zn, Cu, Fe, Cr, and DM were enhanced by the ZnAAC which may be associated with alterations in the intestinal luminal environment.

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CHAPTER V

Comparison of an Inorganic and Organic Zinc Source II. Availability of Zn from an Amino Acid Chelate for Zn Depleted Pigs

Abstract

In three experiments, Zn depleted pigs were fed an isolated soy protein, semipurified diet to compare the availability of Zn from an amino acid chelate (ZnAAC) and $ZnSO_4$. Supplemental levels of Zn used were 5, 15, and 45 ppm in Exp. 1, and 45 ppm in Exp. 2 and 3. Concurrently, Zn adequate pigs were also fed the 45 ppm Zn supplemented diets in Exp. 1 and 2. Depleted pigs fed diets supplemented with 45 ppm Zn either as $ZnSO_4$ or ZnAAC almost completely replenished ($P < .01$) their serum, liver, kidney, pancreas, brain and intestine Zn pools within 6 d. Conversely, depleted pigs fed diets supplemented with either 5 or 15 ppm Zn in Exp. 1 were not able to replenish their Zn stores within a 24-d Zn repletion period. Nevertheless, depleted pigs fed the diets supplemented with 15 ppm Zn performed at a level similar to the performance of the 45 ppm Zn repleted groups. At d 6 in Exp. 2, serum mitogenic activity of the ZnAAC adequate pigs was higher ($P < .05$) compared with the $ZnSO_4$ adequate group. Other indicators of Zn status in both depleted and adequate groups were not affected by Zn source. In most tissues, Cu and Fe contents, like Zn, were not affected by Zn source. Only in the small intestine, Fe contents of depleted and adequate pigs fed ZnAAC were higher ($P < .01$) compared with their respective $ZnSO_4$ groups. In conclusion, the availability of Zn from ZnAAC was apparently not different from that of $ZnSO_4$ when included into a semipurified diet fed to Zn depleted and adequate pigs.

(Key words: pigs, mineral availability, zinc chelate, zinc sulfate, zinc depleted semipurified diet)

Introduction

Recently, industry has propagated the inclusion of organic complexed and chelated Zn products into mineral supplements for livestock diets. They allegedly belief that organic sources of Zn supply more available Zn than commonly used inorganic mineral salts. Improved availability of Zn from the complex Zn methionine compared with $ZnSO_4$ and ZnO was observed in chicks by Wedekind et al. (1992). Furthermore, a slightly improved Zn availability was obtained when Zn methionine instead of ZnO was supplemented to diets of Zn deficient lambs (Spears, 1989). Conversely, substitution of Zn methionine for $ZnSO_4$ in a trace mineral supplement for weanling and growing-finishing diets of pigs did not improve performance (Kornegay and Thomas, 1975; Hill et al, 1986).

Results obtained in studies evaluating the availability of mineral sources must be carefully interpreted. Several factors, other than the mineral source per se, may influence Zn availability. These factors include: (1) the Zn availability of the mineral source used as control (Wedekind et al., 1992; Wedekind and Baker, 1990), (2) interactions of Zn with other dietary constituents (Lönnerdal, 1991), and (3) the Zn status of the experimental animals (Hallmans et al., 1987). The present study was conducted to compare the availability of Zn from an amino acid chelate and from inorganic Zn sulfate when fed to Zn depleted pigs.

Experimental Procedures

Animals, Diets, and Housing. Three experiments were conducted using a total of 60 Zn depleted pigs and 24 Zn adequate pigs. Pigs had been depleted of Zn for 32 d in Exp. 1 and for 24 d in Exp. 2 and 3 by feeding a semipurified diet containing 17 ppm Zn (Table 1). During Zn depletion in Exp. 1 and 2, adequate pigs had been fed the Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or Zn amino acid chelate (Albion Laboratories, Cadco Inc., Des Moines, IA). Pigs were repleted with Zn using the low Zn depletion diet as the basal to which Zn was supplemented either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC).

In Exp. 1, 30 Zn depleted pigs were repleted up to 24 d using the low Zn diet supplemented with 5, 15, or 45 ppm Zn either as $ZnSO_4$ or as ZnAAC. Six randomly selected pigs (one per treatment) were sacrificed on d 0, 3, 6, 12, and 24 by intravenous injection of a lethal dose (.25 mL per kg BW) of sodium pentobarbital (Anthony Products Co., Arcadia CA) followed by exsanguination (Figure 1). Eight adequate pigs (four per group) were continued on the low Zn diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as ZnAAC during the 24-d Zn repletion period and then sacrificed.

In Exp. 2, 32 depleted pigs were assigned to the low Zn diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as ZnAAC and repleted up to 12 d (Figure 1). Eight randomly selected pigs (four per treatment) were sacrificed on d 0, eight pigs (four per treatment) on d 6, and 16 pigs (eight per treatment) on d 12. Concurrently, eight randomly selected Zn adequate pigs (four per group) were

Table 1. Composition of the semipurified diet

Ingredient	(g per kg diet)
Corn starch[a]	336.6
Dextrose[b]	300
Isolated soy protein[c]	200
Corn oil	60
Cellulose[d]	30
Dynafos[e]	29
Mineral mix[f]	16.6
Dyna-K[e]	14.4
$CaCO_3$	5.8
Vitamin mix[g]	4
Dynamate[e]	3.6

[a]Cargill Inc., Minneapolis, MN
[b]Cerelose Dextrose 2001, Corn Products, Summit-Argo, IL
[c]PP500E, Protein Technologies International, St. Louis, MO
[d]Purified powder cellulose BH200, International Filler Co., North Tonawanda, NY
[e]Dynafos contained (minimum) 20% Ca, 18.5% P, and (maximum) .185% F; Dynamate contained (minimum) 22% S, 18% K, and 11% Mg; Dyna-K contained (minimum) 96.5% KCl and 50% K. Pitman-Moore Inc., Mundelein, IL
[f]Provided the following sources of minerals in g per kg of diet: $FeSO_4.7H_2O$, .25; $CuSO_4.5H_2O$, .024; $MnSO_4.H_2O$, .012; KIO_3, .00024; Na_2SeO_3, .00066; dextrose, 15.3
[g]Provided the following amounts per kg of diet: vitamin A, 4,950 IU; vitamin D_3, 660 IU; vitamin E, 33 IU; vitamin K, 6 mg; riboflavin, 3.3 mg; niacin, 35.2 mg; pantothenic acid, 19.8 mg; vitamin B_{12}, .022 mg; choline, 1,184 mg; thiamin, 2.6 mg; vitamin B_6, 3 mg; biotin, .4 mg; folacin, 2mg; D,L-methionine, 1,200 mg

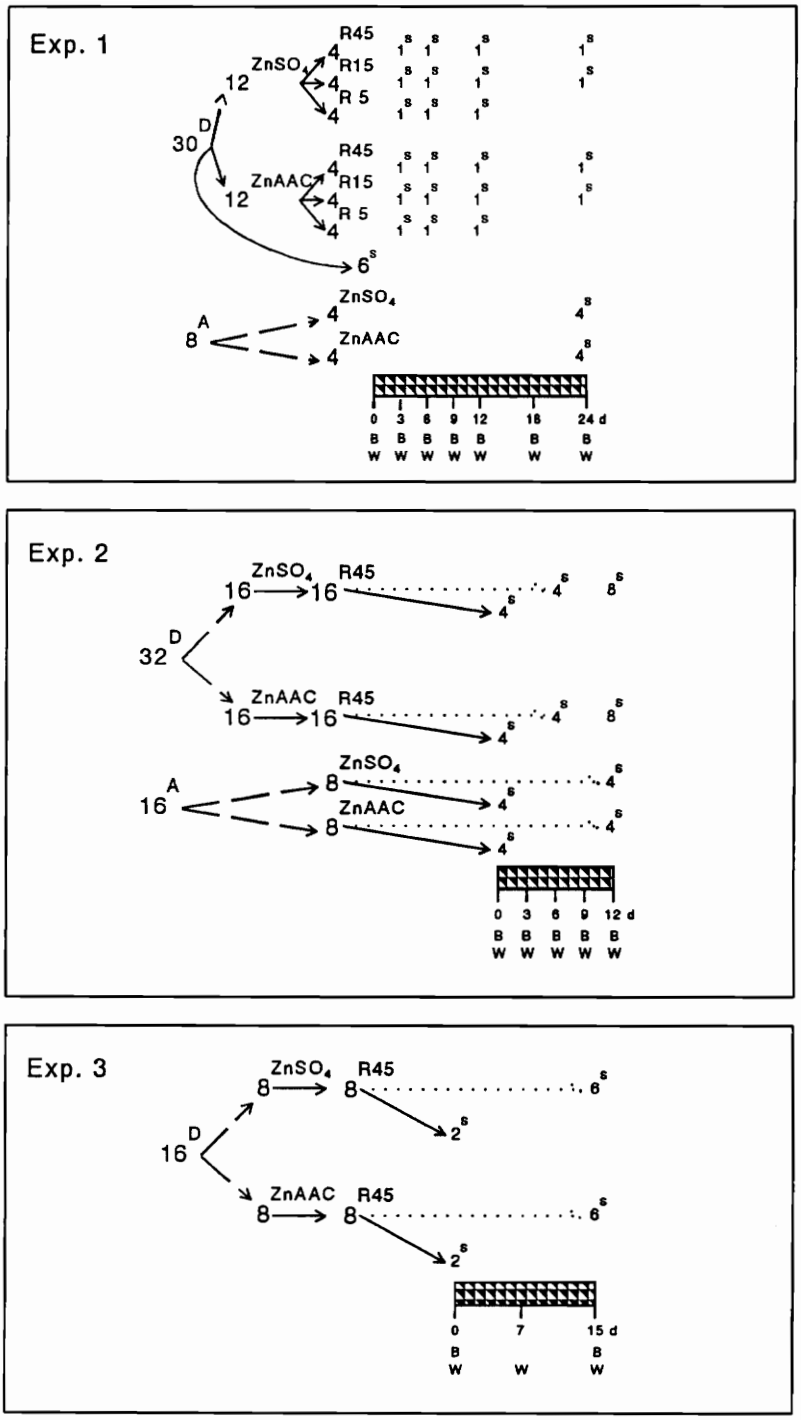

Figure 1. Schematic overview of experimental design and measurements of Exp. 1, 2, and 3. Legend: D = depleted, A = adequate, ZnAAC = Zn amino acid chelate, R# = repleted + level of supplemental Zn, S = sacrificed, B = blood sampled and W = weighed
Zn repletion period

sacrificed on d 0. The other eight adequate pigs (four per group) were continued on the $ZnSO_4$ and ZnAAC diets during the 12-d Zn repletion period and then sacrificed.

In Exp. 3, 16 depleted pigs were assigned to the low Zn diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as ZnAAC (Figure 1). Four depleted pigs (two per treatment) were sacrificed on d 0. The other 12 pigs (six per treatment) were repleted for 15 d and then sacrificed.

In Exp. 1 and 2, pigs were individually housed in 1.5 m x .7 m stainless steel pens (two pigs per pen separated by a wood divider). In Exp. 3, pigs were individually housed in 1.5 m x .5 m stainless steel pens. Pigs were fed twice daily starting at 0600 and 1800. The daily feeding level was 2.5 times maintenance (maintenance = 500 kJ ME per kg $BW^{\cdot 75}$). Pigs were allowed access to the feed for 2 h, after which left over feed was collected and weighed. Deionized water was provided continuously using nipple waterers. An ambient temperature of 23°C and a 14-10 h light-dark cycle was maintained.

Performance, Blood, and Tissue Analysis. Body weight and feed intake were monitored every 3 d for 24 d in Exp. 1 and for 12 d in Exp. 2 (Figure 1). In Exp. 3, BW were taken on d 1, 7, and 15, and feed intake was monitored between the days of weighing. Additionally, blood samples from the anterior vena cava were taken on the days of weighing, except on d 7 in Exp. 3.

All pigs were sacrificed within 2 to 4 h after offering the meal. In all experiments, samples of liver and kidney were collected from each sacrificed pig. Moreover, a sample of the pancreas was collected in Exp. 2. Additionally, the small intestine of all pigs was excised in Exp. 1 and 2, except for the adequate pigs

sacrificed at the end of the 24-d Zn repletion period in Exp. 1. Only the stomach was excised in Exp. 3.

Handling, storage and analyses of serum and tissues were described in Chapter III. In all experiments, serum was analyzed for serum Zn concentration and serum alkaline phosphatase (ALP) activity. On d 6 and 12 in Exp. 2, serum was analyzed for serum mitogenic activity and serum protein concentration. Organ and gastrointestinal (GI) tissues were analyzed for Zn, Cu, and Fe concentrations. Furthermore, DM was determined on all tissue samples.

Statistical Procedures. Performance and serum data were analyzed by the GLM procedure of SAS (1988) using the pig as experimental unit. For data of Exp. 1, the model contained the main effects: (1) Zn status (depleted and adequate), (2) Zn source ($ZnSO_4$ and ZnAAC), and (3) supplemental level of Zn (5, 15, and 45 ppm Zn), and the two-way interaction Zn status x Zn source. Orthogonal contrasts were used to test for linear and quadratic responses to supplemental Zn level. In Exp. 2, the model contained the main effects Zn status and Zn source, and the two-way interaction. In Exp 3, Zn source was the only main effect of the model.

The slopes of the serum Zn and serum ALP-activity data among both depleted and adequate groups were analyzed using the GLM procedure of SAS (1988). The statistical model was similar as the one described in Chapter III, except Diet was replaced by the main effects and two-way interactions used in the models above. Pig was nested within Zn status x Zn source x Zn level in Exp. 1, and within Zn status x Zn source in Exp. 2. Linear and quadratic day by Zn status, day by Zn source, day by Zn level, and day by Zn status X Zn source sequential sums of squares were determined to allow for inferences regarding the similarity of serum Zn concentration and ALP-activity patterns of the depleted and adequate groups.

In Exp. 2 and 3, tissue data of depleted and adequate pigs were analyzed by the GLM procedure of SAS (1988). The analysis of tissue data in Exp. 2 involved two steps. Initially, only data obtained on d 0 and 12 were analyzed. The model contained the main effects Zn status, Zn source and day, and all two- and three-way interactions. The Zn status x day interaction ($P < .05$) was used to identify the tissue variables that differed in their pattern between the depleted and adequate pigs. Afterwards, tissue data were analyzed separately for the depleted and adequate pigs. In this analysis, tissue data of depleted pigs sacrificed on d 6 were included. Statistical models of both depleted and adequate groups contained Zn source and day as main effects and the two-way interaction. For the depleted pigs, orthogenal contrasts were used to test for linear and quadratic responses to day. In Exp. 3, tissue data of the depleted pigs were analyzed as described for the adequate pigs in Exp. 2.

Results

General. In Exp. 1, Zn depleted pigs fed the low Zn diet with 5 ppm Zn either as $ZnSO_4$ or ZnAAC lost weight and further depleted their serum and tissue Zn stores. After 12 d of Zn repletion, both 5 ppm Zn groups were taken off study because of poor condition. Only serum Zn levels, serum ALP-activities and performance data of the 5 ppm Zn groups are presented (Table 2 and Figure 2).

Dietary concentrations of the low Zn depletion diet were 17 ppm Zn, 16 ppm Cu and 480 ppm Fe. The $ZnSO_4$ diets were analyzed and found to contain 26 and 64 ppm Zn and the ZnAAC diets 34 and 66 ppm Zn, respectively, for the low Zn diets supplemented with 15 and 45 ppm Zn.

Table 2. Performance of Zn depleted pigs fed the low Zn depletion diet supplemented with 5, 15, and 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC), and of Zn adequate pigs fed the low Zn diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as ZnAAC in Exp. 1[a]

	Depleted[b]						Adequate[b]		
	$ZnSO_4$			ZnAAC			$ZnSO_4$	ZnAAC	
Item	5	15	45	5	15	45			SEM
D 1 - 3									
ADG, kg	.03	.09	.15	.04	.09	.08	.49	.48	.05[c]
ADFI, kg	.28	.34	.30	.28	.30	.32	.65	.64	.03[c]
Gain:feed	.13	.21	.49	.17	.33	.22	.75	.76	.14[d]
D 4 - 24									
ADG, kg	-.03	.45	.49	-.03	.29	.47	.54	.50	.06[de]
ADFI, kg	.35	.56	.62	.27	.45	.53	.83	.80	.04[ce]
Gain:feed	NC[f]	.81	.80	NC[f]	.63	.91	.65	.63	.15[g]
D 1 - 24[h]									
ADG, kg	.01	.26	.32	.01	.21	.31	.53	.50	.06[cg]
ADFI, kg	.31	.45	.46	.27	.37	.44	.81	.78	.06[cg]
Gain:feed	.08	.52	.68	.04	.55	.71	.66	.64	.10[g]

[a]At the start of repletion, mean BW of the depleted pigs fed $ZnSO_4$ and ZnAAC were, respectively, 13.1 $\pm$ .8 and 11.7 $\pm$ .8 kg. Starting BW of the Zn adequate pigs fed $ZnSO_4$ and ZnAAC were, respectively, 15.8 $\pm$ .5 and 15.2 $\pm$ .5 kg

[b]Each depleted mean represents four pigs initially with one pig sacrificed on d 3, 6, and 12. Each adequate mean represents four pigs

[c,d]Zn status effect, respectively, ($P < .01$) and ($P < .05$)

[e,g]Linear and quadratic increase with level of Zn, respectively, ($P < .05$) and ($P < .01$)

[f]Not Computed

[h]Zn depleted pigs repleted with 5 ppm Zn either as $ZnSO_4$ or as ZnAAC were taken off study at 12 d

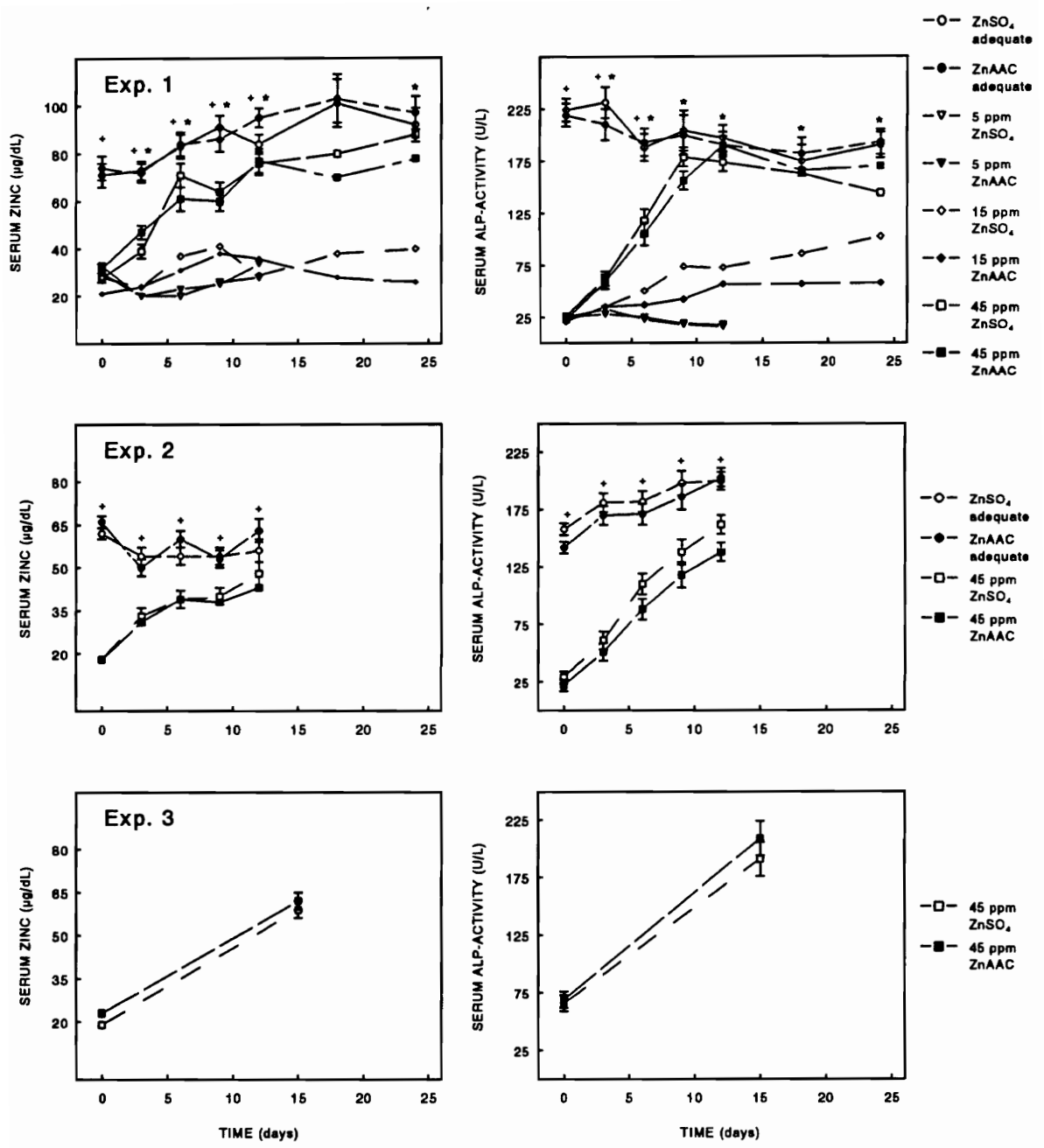

Figure 2. Serum Zn concentrations and serum alkaline phosphatase (ALP) activity of Zn depleted and Zn adequate pigs fed the low Zn depletion diet with 5, 15, or 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) in Exp. 1, 2, and 3 Each depleted mean represents initially four pigs in Exp. 1 with one pig sacrificed on d 3, 6, and 12, initially 12 pigs in Exp. 2 with four pigs sacrificed on d 6, and six pigs in Exp. 3. Each adequate mean represents four pigs
+Zn status effect ($P < .05$), *Linear increase with level of Zn ($P < .05$)

Serum Characteristics and Performance during Zn Repletion. Analyses of slopes of serum Zn concentration and serum ALP-activity showed interactive ($P < .05$) day x Zn status and day x Zn level effects, whereas, the day x Zn source interaction was non-significant. Serum Zn levels and serum ALP-activity of depleted pigs fed the 45 ppm $ZnSO_4$ and ZnAAC diets rapidly increased, respectively, to d 6 and 12 (Figure 2), and then gradually increased to values which approximated those of the adequate groups. Conversely, increases in both serum Zn levels and serum ALP-activity of pigs repleted with 15 ppm Zn were smaller ($P < .05$), and even decreased ($P < .05$) for depleted groups fed the 5 ppm $ZnSO_4$ and ZnAAC diets.

A slight increase in serum Zn concentration was observed for both adequate groups in Exp. 1, whereas, serum ALP-activities slightly decreased throughout the 24-d Zn repletion period. Conversely, serum ALP-activities were slightly increased and serum Zn levels were maintained for the adequate groups in Exp. 2.

During Zn repletion in Exp. 2, serum mitogenic activity of the depleted pigs fed $ZnSO_4$ and ZnAAC apparently increased up to d 6 (Figure 3). After 6 d, the serum mitogenic activity of the adequate pigs fed ZnAAC was higher ($P < .05$) compared with the adequate $ZnSO_4$ group. The trends found in serum mitogenic activity were not associated with differences in serum protein concentrations (Figure 3). The depleted and adequate groups maintained similar serum protein levels throughout the entire Zn repletion period.

Due to a high BW, adequate pigs fed 45 ppm Zn either as $ZnSO_4$ or as ZnAAC were offered a larger amount of feed than depleted pigs fed the Zn repletion diets (see footnotes of Tables 2 and 3). Consequently, overall ADG of the $ZnSO_4$ and ZnAAC adequate groups was higher ($P < .01$) than of their respective

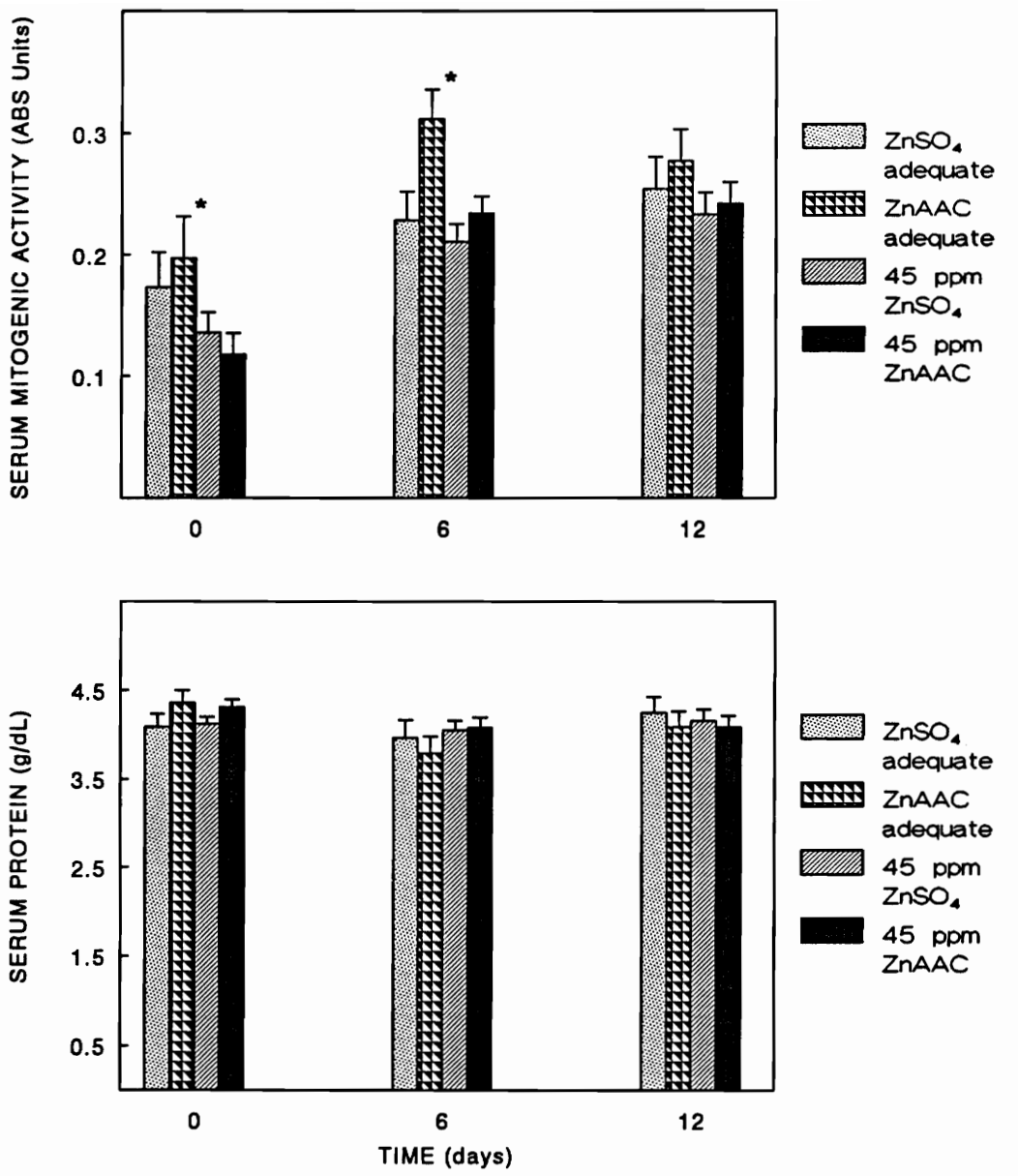

Figure 3. Total serum mitogenic activity and total serum protein concentration of Zn depleted and Zn adequate pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) in Exp. 2 Each depleted mean represent 12 pigs initially with four pigs sacrificed on d 6. Each adequate mean represents four pigs. At d 0 and 6, *Zn status effect ($P < .05$) and at d 6, Zn status by Zn source interaction ($P < .05$)

Table 3. Performance of Zn depleted and Zn adequate pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) in Exp. 2 and 3[a]

	Depleted[b]		Adequate[b]		
Item	$ZnSO_4$	ZnAAC	$ZnSO_4$	ZnAAC	SEM
Exp. 2, D 1 - 3					
ADG, kg	.10	.13	.30	.29	.04[c]
ADFI, kg	.26	.29	.41	.41	.02[c]
Gain:feed	.60	.56	.66	.66	.04[d]
D 4 - 12					
ADG, kg	.28	.30	.37	.39	.02[c]
ADFI, kg	.44	.48	.59	.60	.02[c]
Gain:feed	.65	.62	.64	.65	.04
D 1 - 12					
ADG, kg	.22	.24	.36	.37	.03[c]
ADFI, kg	.38	.42	.54	.56	.02[c]
Gain:feed	.55	.58	.66	.66	.05
Exp. 3, D 1 - 15					
ADG, kg	.29	.28			.03
ADFI, kg	.42	.42			.03
Gain:feed	.70	.65			.02

[a]At the start of Zn repletion, mean BW of the Zn depleted pigs fed $ZnSO_4$ and ZnAAC were, respectively, 13.1 $\pm$ .8 and 11.7 $\pm$ .8 kg in Exp. 2, and 10.1 $\pm$ .8 and 9.9 $\pm$ .8 kg in Exp. 3. Starting BW of Zn adequate pigs fed $ZnSO_4$ and ZnAAC were, respectively, 15.8 $\pm$ .5 and 15.2 $\pm$ .5 kg

[b]In Exp. 2, each depleted mean represents twelve pigs initially with four pigs sacrificed on d 6, and each adequate mean four pigs. In Exp. 3, each depleted mean represents six pigs

[c,d]Zn status effect, respectively, ($P < .01$) and ($P < .05$)

depleted groups. Between d 1 and 3, the higher ($P < .01$) ADG of the adequate pigs was associated with a higher ($P < .05$) GF-ratio compared with the depleted pigs fed the low Zn diet supplemented with 5, 15, or 45 ppm Zn. From d 4 to 24 in Exp. 1, however, GF-ratios of depleted pigs fed the low Zn diet supplemented with 15 ppm and 45 ppm $ZnSO_4$, or 45 ppm ZnAAC were numerically higher compared with the adequate pigs. In Exp. 2, GF-ratios of the depleted and adequate groups were similar throughout the 12-d Zn repletion period. In all three experiments, performance of either the depleted or adequate pigs was not affected by Zn source.

Organ Tissue Weights. In Exp. 2 and 3, liver and kidney weights, expressed as a % of BW, of depleted and adequate groups were similar throughout the Zn repletion periods (Table 4). In Exp. 2, relative pancreas weight of depleted and adequate pigs fed $ZnSO_4$ and ZnAAC increased ($P < .01$) during Zn repletion, but the increase was more profound ($P < .05$) for the depleted pigs. Moreover at d 6, pancreas weight of the depleted pigs fed ZnAAC was higher ($P < .05$) compared with the $ZnSO_4$ depleted group. A decrease ($P < .01$) in relative brain weights was found for both depleted and adequate pigs fed $ZnSO_4$ or ZnAAC.

Zinc, Cu, and Fe Concentrations in Organ Tissues. In Exp. 2 and 3, depleted pigs fed 45 ppm Zn either as $ZnSO_4$ or ZnAAC increased ($P < .01$) the concentrations of Zn in all organ tissues (Tables 5 and 8). Apparent increases in liver and kidney Zn contents also were observed for the depleted groups fed 45 ppm Zn, but not for the 15 ppm Zn repleted groups, in Exp. 1 (Figure 4). Liver and brain Zn contents of the depleted groups in Exp. 2 increased ($P < .01$) up to d 12, whereas, kidney and pancreas Zn levels only increased ($P < .01$) up to d 6 (Table 5). After 6 d, kidney Zn levels plateaued ($P < .05$) and pancreas Zn levels decreased ($P < .05$). The magnitude of Zn accumulation was greatest in the liver and pancreas.

Table 4. Wet weights, expressed as % of BW, of organ tissues of Zn depleted pigs fed the low Zn depletion diet supplemented with 15 and 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC), and of Zn adequate pigs fed the low Zn diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as ZnAAC in Exp. 1 and 2

	Depleted[a]					Adequate[a]		
	$ZnSO_4$		ZnAAC			$ZnSO_4$	ZnAAC	
Tissue	15	45	15	45	SEM			SEM
Exp. 1[b]								
Liver	2.7	2.5	2.6	3.0	.21	2.2	2.3	.18
Kidney	.24	.24	.27	.25	.02	.18	.17	.01
Exp. 2								
Liver								
Day 0		2.1		2.5		2.5	2.1	
6		2.5		2.8				
12		2.7		2.9	.15[cd]	2.6	2.6	.20
Kidney								
Day 0		.23		.24		.23	.23	
6		.27		.23				
12		.25		.25	.02	.22	.22	.01
Pancreas[e]								
Day 0		.12		.11		.14	.14	
6		.15		.23				
12		.21		.20	.01[df]	.18	.20	.01[g]

Table 4 (continued)

Brain							
Day	0	.61	.50		.46	.49	
	6	.52	.46				
	12	.42	.39	.03[hi]	.36	.34	.03[j]
Exp. 3							
Liver							
Day	0	2.5	2.5				
	15	2.3	2.3	.11			
Kidney							
Day	0	.22	.21				
	15	.21	.21	.01			

[a]In Exp. 1, each depleted mean represents four pigs, sacrificed on d 3, 6, 12, and 24 (one pig per day), and each adequate mean four pigs, sacrificed on d 24. In Exp. 2, each depleted mean represents four pigs on d 0 and 6, and eight pigs on d 12. Each adequate mean represents four pigs. In Exp. 3, each depleted mean represents two pigs on d 0 and six pigs on d 15

[b]On d 0, liver and kidney weights of six depleted pigs were, respectively, 2.9 $\pm$ .14 and .27 $\pm$ .02 %

[c,h]Zn source effect, respectively, ($P < .05$) and ($P < .01$)

[d]Linear increase with day ($P < .01$)

[e]Zn status by day interaction ($P < .05$)

[f]Zn source by day interaction ($P < .05$)

[g]Increase with day ($P < .01$)

[i]Linear decrease with day ($P < .01$)

[j]Decrease with day ($P < .01$)

Table 5. Zinc concentrations in organ tissues of Zn depleted and Zn adequate pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) in Exp. 2

Organ tissue			Depleted[a]			Adequate[a]		
			$ZnSO_4$	ZnAAC	SEM	$ZnSO_4$	ZnAAC	SEM
			ppm, DM basis					
Liver[b]	D	0	150	143		264	218	
		6	203	173				
		12	215	216	14[c]	279	221	28[d]
Kidney[b]	D	0	135	130		163	158	
		6	143	151				
		12	143	150	5.9[e]	159	153	5.8
Pancreas[b]	D	0	132	122		194	195	
		6	163	190				
		12	141	140	9.4[f]	149	149	10[g]
Brain[b]	D	0	86	85		95	94	
		6	88	91				
		12	91	90	1.4[c]	89	93	1.5[h]

[a]On d 0 and 6, each depleted mean and each adequate mean represent four pigs. On d 12, each depleted mean represents eight pigs and each adequate mean four pigs
[b]Zn status by day interaction ($P < .05$)
[c]Linear increase with day ($P < .01$)
[d]Zn source effect ($P < .10$)
[e]Linear and quadratic increase with day ($P < .05$)
[f]Quadratic increase with day ($P < .01$)
[g]Decrease with day ($P < .01$)
[h]Decrease with day ($P < .10$)

Table 6. Copper concentrations in organ tissues of Zn depleted and Zn adequate pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) in Exp. 2

Organ tissue			Depleted[a]			Adequate[a]		
			$ZnSO_4$	ZnAAC	SEM	$ZnSO_4$	ZnAAC	SEM
			ppm, DM basis					
Liver	D	0	94	99		112	98	
		6	67	46				
		12	67	66	13[b]	48	48	7.9[c]
Kidney[d]	D	0	27	25		65	56	
		6	34	45				
		12	43	55	4.7[ef]	52	48	9.2
Pancreas[d]	D	0	8.2	7.2		8.4	8.4	
		6	10.2	9.5				
		12	10.1	9.2	.6[g]	9.1	9.0	.3[h]
Brain	D	0	27	27		28	27	
		6	28	31				
		12	28	29	1.3	26	30	9[i]

[a]On d 0 and 6, each depleted mean and each adequate mean represent four pigs. On d 12, each depleted mean represents eight pigs and each adequate mean four pigs

[b]Linear and quadratic decrease with day ($P < .05$)

[c]Decrease with day ($P < .01$)

[d]Zn status by day interaction ($P < .05$)

[e]Linear increase with day ($P < .05$)

[f]Zn source effect ($P < .10$)

[g]Linear and quadratic increase with day ($P < .05$)

[h]Increase with day ($P < .10$)

[i]Zn source by day interaction ($P < .10$)

Table 7. Iron concentrations in organ tissues of Zn depleted and Zn adequate pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) in Exp. 2

Organ tissue		Depleted[a]			Adequate[a]		
		$ZnSO_4$	ZnAAC	SEM	$ZnSO_4$	ZnAAC	SEM
		ppm, DM basis					
Liver	D 0	529	224		211	537	
	6	297	275				
	12	134	134	50[bcd]	169	178	56[cde]
Kidney	D 0	357	277		307	319	
	6	312	278				
	12	233	271	31[b]	244	247	21[e]
Pancreas	D 0	116	85		95	121	
	6	145	113				
	12	107	108	19	106	113	9.5
Brain	D 0	87	86		84	87	
	6	95	94				
	12	94	90	3.5	100	102	5.1[f]

[a]On d 0 and 6, each depleted mean and each adequate mean represent four pigs. On d 12, each depleted mean represents eight pigs and each adequate mean four pigs

[b]Linear decrease with day ($P < .05$)

[c]Zn source effect ($P < .05$)

[d]Zn source by day interaction ($P < .05$)

[e]Decrease with day ($P < .01$)

[f]Increase with day ($P < .01$)

Table 8. Zinc, Cu and Fe concentrations (ppm), expressed on a DM basis, in organ tissues of Zn depleted pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) in Exp. 3

Organ tissue			Depleted[a]		SEM
			$ZnSO_4$	ZnAAC	
			-----Zinc-----		
Liver	Day	0	151	171	
		15	252	200	15[bc]
Kidney	Day	0	151	147	
		15	174	163	4.1[b]
			----Copper----		
Liver	Day	0	162	134	
		15	122	143	28
Kidney	Day	0	31	36	
		15	53	46	6.5[d]
			-----Iron-----		
Liver	Day	0	526	383	
		15	303	333	94
Kidney	Day	0	175	171	
		15	206	224	15[e]

[a]On d 0, each depleted mean represents two pigs and on d 15 six pigs
[b]Increase with day ($P < .01$)
[c]Zn source by day interaction ($P < .10$)
[d,e]Increase with day, respectively, ($P < .10$) and ($P < .05$)

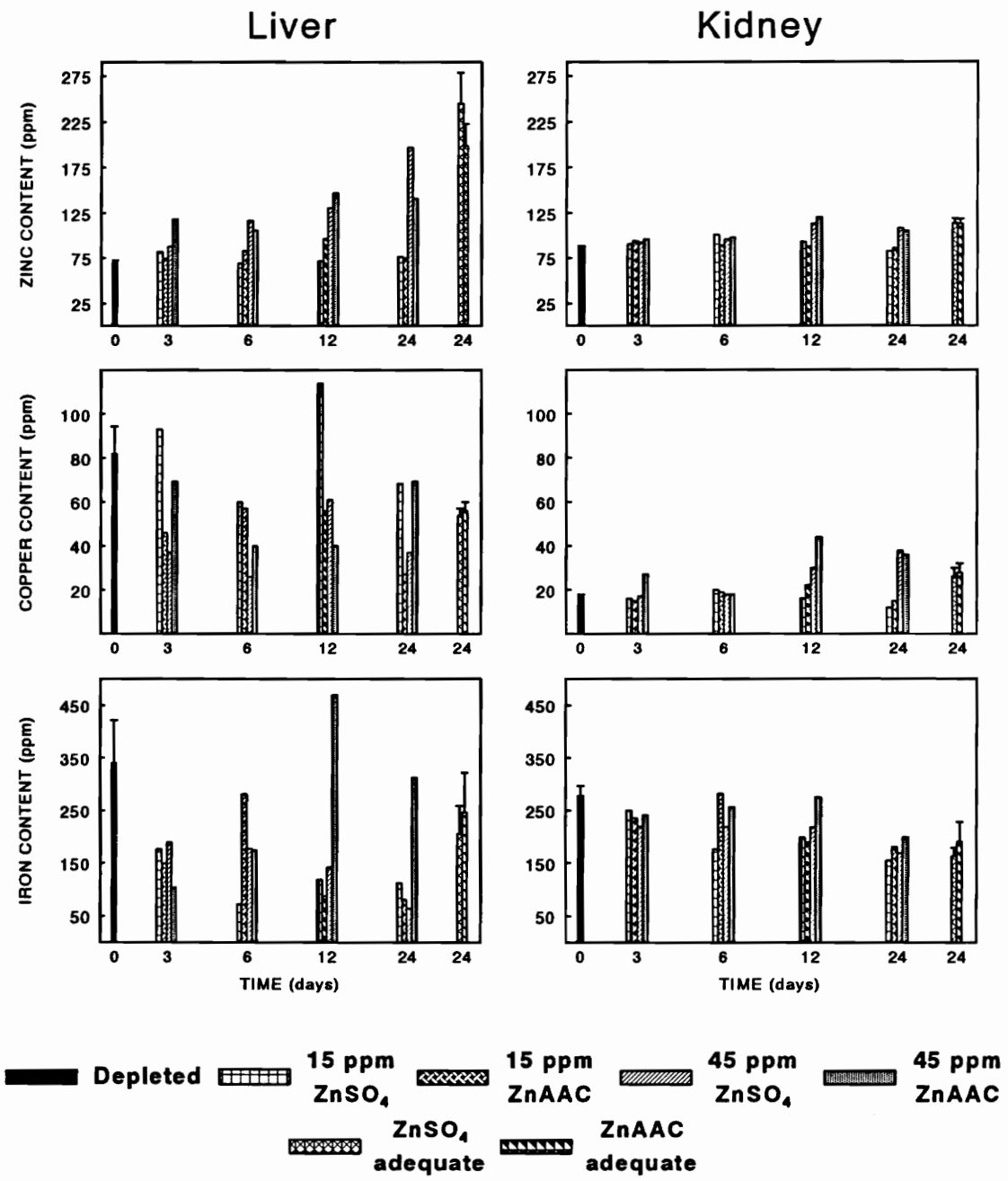

Figure 4. Zinc, Cu, and Fe contents, expressed on a DM basis, in liver and kidney of Zn depleted and Zn adequate pigs fed the low Zn depletion diet, or the low Zn diet supplemented with 15 or 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) in Exp. 1. On d 0, each depleted mean represents six pigs. On d 3, 6, 12, and 24 each depleted value represents one pig. Each adequate mean represents four pigs

Conversely, adequate pigs maintained their liver, kidney, and brain tissue Zn contents, and decreased ($P < .01$) their pancreas Zn levels during Zn repletion. Tissue Zn levels attained by the depleted pigs fed $ZnSO_4$ and ZnAAC were just below those of their respective adequate groups. Levels of tissue Zn were not clearly affected by Zn source, except for depleted pigs fed $ZnSO_4$ in both Exp. 2 and 3, where liver Zn concentrations tended to be higher ($P < .10$) compared with the depleted pigs fed ZnAAC.

During Zn repletion in Exp. 2 and 3, kidney Cu levels of the depleted groups increased ($P < .01$), but were maintained for the adequate fed pigs (Tables 6 and 8). Pancreas Cu levels increased ($P < .10$) for both depleted and adequate pigs, although more profound ($P < .05$) for the depleted pigs. Conversely, liver Cu, and liver and kidney Fe levels (Tables 7 and 8) mostly decreased ($P < .01$) for both depleted and adequate pigs. Brain Cu, and Fe levels in the brain and pancreas of depleted and adequate pigs were maintained, except for an increase ($P < .01$) in brain Fe content for adequate pigs fed ZnAAC. Trends in tissue Cu and Fe levels observed for the depleted pigs in Exp. 2 and 3 were also observed for the depleted pigs fed the 45 ppm $ZnSO_4$ and ZnAAC diets in Exp. 1 (Figure 4). In all experiments, tissue Cu and Fe concentrations were similar for depleted or adequate pigs fed the $ZnSO_4$ and ZnAAC diets.

Zinc, Cu, and Fe Concentrations in GI Tissues. In Exp. 2, intestinal Zn concentrations increased ($P < .05$) in the proximal and medial segments of the small intestine of depleted pigs up to d 12 (Table 9). Conversely for the adequate pigs, a decrease in intestinal Zn content ($P < .01$) was found in both proximal and medial segments of the small intestine. Intestinal Zn levels in the distal small intestine segment tended to decrease ($P < .10$) for both depleted and adequate groups.

Table 9. Zinc concentrations in gastrointestinal (GI) tissues of Zn depleted and Zn adequate pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) in Exp. 2 and 3

GI tissue[b]			Depleted[a]			Adequate[a]		
			$ZnSO_4$	ZnAAC	SEM	$ZnSO_4$	ZnAAC	SEM
			ppm, DM basis					
Proximal[c]	D	0	116	122		134	139	
		6	135	130				
		12	131	129	3.6[d]	133	130	2.2[ef]
Medial[c]	D	0	127	124		144	136	
		6	123	128				
		12	132	131	2.4[g]	129	133	2.8[ef]
Distal	D	0	156	138		159	158	
		6	157	147				
		12	135	137	5.9[hi]	137	138	4.2[j]
Stomach	D	0	132	132				
		15	130	119	8.1			

[a]In Exp. 2, each depleted mean represents four pigs on d 0 and 6, and eight pigs on d 12. Each adequate mean represents four pigs on d 0 and 12. In Exp. 3, each depleted mean represents two pigs on d 0, and six pigs on d 15
[b]GI segments were the proximal, medial and distal segment of the small intestine in Exp. 2, and the stomach in Exp. 3
[c]Zn status by day interaction ($P < .05$)
[d]Linear and quadratic increase with day ($P < .05$)
[e,j]Decrease with day, respectively, ($P < .05$) and ($P < .10$)
[f]Zn source by day interaction ($P < .10$)
[g]Linear increase with day ($P < .05$)
[h]Linear and quadratic decrease with day ($P < .10$)
[i]Zn source effect ($P < .10$)

During Zn repletion in Exp. 3, stomach Zn concentrations of the depleted pigs were maintained (Table 9). Trends of intestinal mucosal Zn levels of depleted pigs in Exp. 1 were similar to those observed in intestinal Zn levels of depleted pigs in Exp. 2 (Figure 5). Both intestinal mucosa and whole intestinal Zn concentrations increased from the proximal towards the distal end of the small intestine and, like Zn levels in the stomach, were not affected by Zn source.

In Exp. 2, intestinal Cu concentrations of depleted pigs increased ($P < .05$) in the proximal segment and decreased ($P < .05$) in the medial and distal segments of the small intestine (Table 10). Adequate pigs also had decreased ($P < .05$) intestinal Cu levels in the medial segment, but maintained their intestinal Cu levels in the proximal and distal segment of the small intestine. Patterns of intestinal Fe concentrations were different ($P < .05$) between the depleted and adequate groups (Table 11). This was primarily due to extremely high ($P < .05$) intestinal Fe levels in the proximal and medial small intestine segments of depleted and adequate pigs fed ZnAAC compared with $ZnSO_4$. A similar accumulation of Fe also was observed in small intestinal mucosa of depleted pigs fed 15 or 45 ppm ZnAAC in Exp. 1 (Figure 5), but not in stomach tissue of depleted pigs fed ZnAAC in Exp. 3 (Table 11).

Discussion

The Zn depleted pigs used in this study were severely Zn deficient. Before the start of Zn repletion, Zn concentrations in body fluids and tissues of the Zn depleted pigs were 10 to 60 % below those of pair-fed controls (Chapter III). All pigs were affected by parakeratosis, the classical sign of Zn deficiency (Tucker and

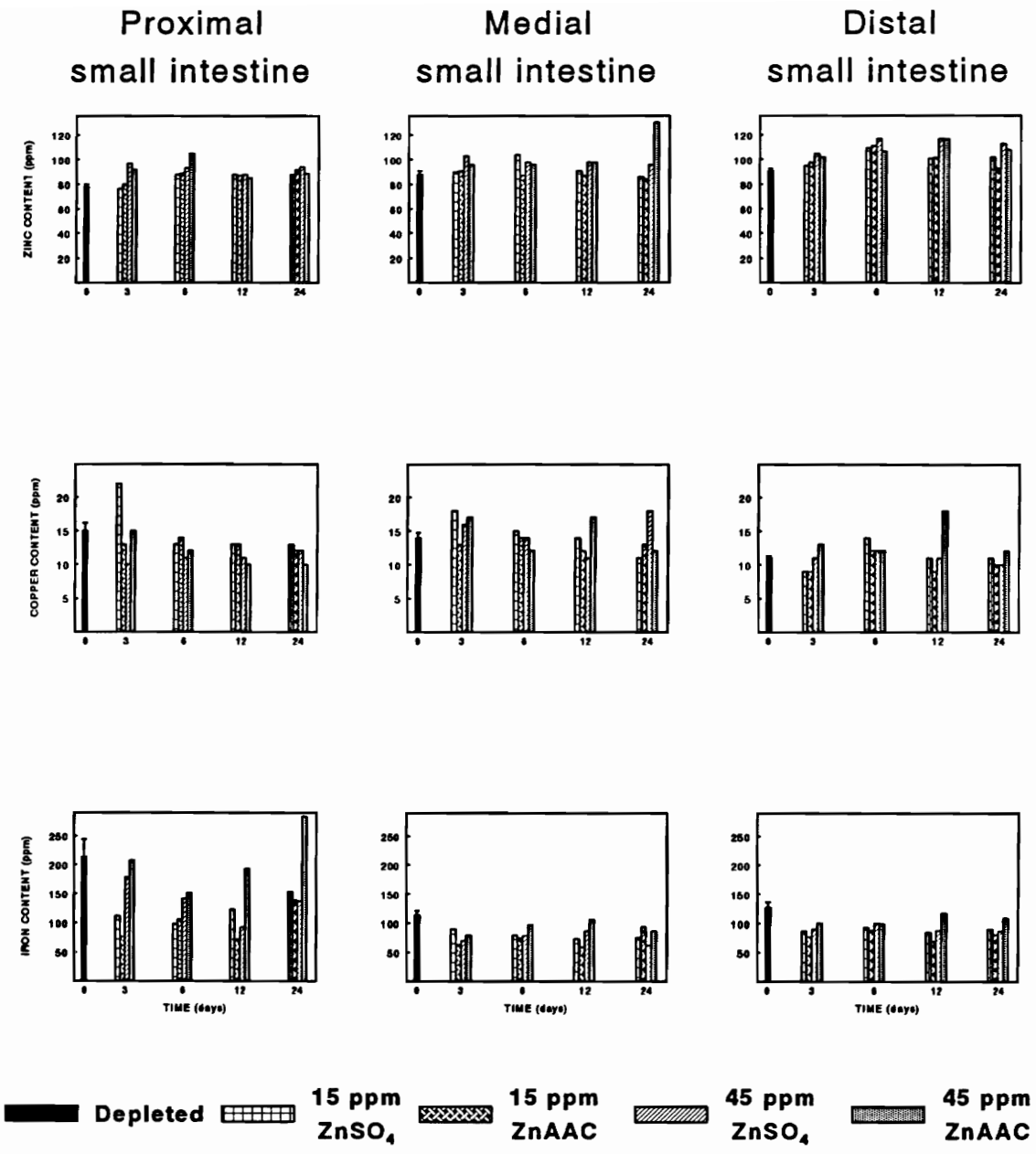

Figure 5. Zinc, Cu, and Fe contents, expressed on a DM basis, in mucosa of proximal, medial and distal segments of the small intestine of Zn depleted pigs fed the low Zn depletion diet supplemented with 15 and 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) in Exp. 1. On d 0, each depleted mean represents six pigs and on d 3, 6, 12, and 24, each depleted value represents one pig

Table 10. Copper concentrations in gastrointestinal (GI) tissues of Zn depleted and Zn adequate pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) in Exp. 2 and 3

GI tissue[b]			Depleted[a]			Adequate[a]		
			$ZnSO_4$	ZnAAC	SEM	$ZnSO_4$	ZnAAC	SEM
			ppm, DM basis					
Proximal[c]	D	0	23	23		17	15	
		6	19	17				
		12	18	18	1.4[d]	16	15	1.3
Medial	D	0	19	19		18	15	
		6	16	15				
		12	13	14	2.0[e]	13	13	1.1[f]
Distal	D	0	12	10		12	13	
		6	12	12				
		12	16	14	1.6[g]	12	15	.7[hi]
Stomach	D	0	14	15				
		15	16	14	1.0			

[a]In Exp. 2, each depleted mean represents four pigs on d 0 and 6, and eight pigs on d 12. Each adequate mean represents four pigs on d 0 and 12. In Exp. 3, each depleted mean represents two pigs on d 0, and six pigs on d 15

[b]GI segments were the proximal, medial and distal segment of the small intestine in Exp. 2, and the stomach in Exp. 3

[c]Zn status by day interaction ($P < .05$)

[d,e]Linear decrease with day, respectively, ($P < .01$) and ($P < .05$)

[f]Decrease with day ($P < .05$)

[g]Linear increase with day ($P < .05$)

[h]Zn source effect ($P < .05$)

[i]Zn source by day interaction ($P < .10$)

Table 11. Iron concentrations in gastrointestinal (GI) tissues of Zn depleted and Zn adequate pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) in Exp. 2 and 3

GI tissue[b]			Depleted[a]			Adequate[a]		
			$ZnSO_4$	ZnAAC	SEM	$ZnSO_4$	ZnAAC	SEM
			ppm, DM basis					
Proximal[c]	D	0	135	114		151	223	
		6	117	177				
		12	100	147	13[def]	137	158	15[eg]
Medial	D	0	87	78		91	100	
		6	62	90				
		12	59	75	6.1[efh]	79	86	6.7[i]
Distal	D	0	96	78		89	97	
		6	77	77				
		12	63	80	6.0	75	95	4.2[ej]
Stomach	D	0	91	74				
		15	90	91	7.9			

[a]In Exp. 2, each depleted mean represents four pigs on d 0 and 6, and eight pigs on d 12. Each adequate mean represents four pigs on d 0 and 12. In Exp. 3, each depleted mean represents two pigs on d 0, and six pigs on d 15
[b]GI segments were the proximal, medial and distal segment of the small intestine in Exp. 2, and the stomach in Exp. 3
[c]Zn status by day interaction ($P < .10$)
[d]Quadratic increase with day ($P < .10$)
[e,j]Zn source effect, respectively, ($P < .05$) and ($P < .01$)
[f]Zn source by day interaction ($P < .05$)
[g,i]Decrease with day, respectively, ($P < .05$) and ($P < .10$)
[h]Linear decrease with day ($P < .05$)

Salmon, 1955). Thus, Zn depleted pigs fed the $ZnSO_4$ and ZnAAC diets had a high body need for Zn.

Replenishment of Body Fluid and Tissue Zn Pools. In Exp. 1, supplemental levels of 5 and 15 ppm Zn, which provided in total 22 and 32 ppm dietary Zn, respectively, were not sufficient to meet the Zn requirement (42 ppm Zn) determined in weanling pigs fed an isolated soy protein, semipurified diet (Smith et al., 1961). Although pigs repleted with 15 ppm supplemental Zn either as $ZnSO_4$ or as ZnAAC were not able to replenish a significant proportion of their body Zn stores, they regained their appetite and their ability to grow (Table 3). Apparently, pigs repleted with 15 ppm Zn were able to replenish body Zn pools that are essential for stimulation of feed intake and growth.

Depleted pigs fed diets supplemented with 45 ppm Zn either as $ZnSO_4$ or ZnAAC were able to replenish most of their body Zn pools within 6 d. A fast replenishment of blood and tissue Zn stores was also observed in Zn deficient guinea-pigs and rats fed diets containing Zn levels which met or where in excess of their Zn requirement (Canton and Cremin, 1990; Dørup et al., 1991; Gupta et al., 1989; Roth and Kirchgessner, 1974). After 6 d, a slow rate of Zn accumulation was only observed in serum and liver.

In contrast to serum and tissue concentrations of Zn, serum ALP-activities increased to d 12 of Zn repletion. It may have been that the increase in serum ALP-activity was indicative of improvement in Zn status which would suggest that body Zn pools were not completely repleted. A further indication of ongoing improvement of Zn status may have been the observation that serum and tissue Zn concentrations attained by depleted pigs fed $ZnSO_4$ and ZnAAC were below those of their respective adequate groups. Alternatively, tissue Zn stores of adequate pigs

may have been higher because they were able to sequester a small amount of Zn during the Zn depletion and repletion periods. Tissue levels of Zn as well as the magnitude of Zn replenishment suggest that pigs depleted for 32 d in Exp. 1 were more Zn deficient than pigs depleted for 24 d in Exp. 2 and 3.

Growth Recovery of the Zn Depleted Pigs. It took approximately 3 d for the Zn depleted pigs to regain their appetite and grow (Tables 2 and 3). The high serum growth factor activity of the adequate pigs fed ZnAAC (Figure 3) was not associated with growth differences between the ZnAAC and $ZnSO_4$ groups. The feeding level of all pigs was 2.5 times maintenance. Therefore, it may be that the adequate pigs fed ZnAAC were offered insufficient amounts of feed to fully exploit their potential for growth.

Bioavailability of Zn from $ZnSO_4$ and ZnAAC. Organic chelated or complexed forms of Zn may improve Zn availability by preventing Zn from binding to non-absorbable dietary compounds (Kratzer and Vohra, 1986; Lönnerdal, 1991). Alternatively, the ligand may directly facilitate Zn absorption as shown in a Zn absorption model proposed by Cousins (1989).

Our findings suggest that the availability of Zn was not different between ZnAAC and $ZnSO_4$. This does not imply that we can exclude a potential beneficial effect of ZnAAC in diets containing factors which negatively affect Zn absorption. Feedstuffs used in the semipurified diet were of high quality and contained at most only small amounts of dietary fiber or anti-nutritional factors such as phytic acid.

Since only absorbed nutrients that are utilized in a biological process can be considered bioavailable (O'Dell, 1984), availability of Zn can be improved by increasing Zn absorption and(or) enhancing Zn retention. In Exp. 3, apparent absorption coefficients of Zn varied when compared between the indirect indicator

method and the balance (Chapter IV). A lack of improvement in apparent Zn absorption, as indicated by the indicator data of Exp. 3, is supported by the bioavailability data of this study. Enhancement of Zn absorption by ZnAAC, as indicated by the balance data of Exp. 3, is only possible when tissue specific accumulation of Zn occurs. Neither liver, kidney, pancreas, brain and intestine tissue Zn levels nor urinary excretions of Zn were affected by the Zn source (Chapter IV). However, it should be pointed out that among other tissues, bones which are often evaluated in mineral availability studies (Hill et al., 1986; Wedekind et al., 1992) were not examined in our study.

Systemic Zn, Cu, and Fe Interactions. Systemic interactions between Zn and Cu are generally believed to involve metallothionein (Cousins, 1985) which is synthesized in the liver, kidney, pancreas and intestine (Bremner and May, 1989). The increase in kidney Cu content (Table 6) of depleted pigs observed during Zn repletion may be associated with stimulation of metallothionein synthesis by Zn, because metallothionein has a higher affinity for Cu than Zn (Dunn et al., 1987). Recently, it has been shown that with increased dietary Zn intake induction of metallothionein synthesis occurred primarily in the kidney (Cousins and Lee-Ambrose, 1992).

The high Fe level found in the intestinal mucosa and whole intestine (Table 11 and Figure 5) of depleted and adequate pigs fed ZnAAC may account for the improved Fe absorption found in depleted pigs fed ZnAAC in Exp. 3 (Chapter IV). Since intestinal Zn and Cu levels were not enhanced in pigs fed the ZnAAC diets, a specific interaction seems to occur between Fe and the ZnAAC. Iron homeostasis is believed to be regulated in the enterocyte involving ferritin and transferrin (Savin and Cook, 1980). Ferritin has been implicated to regulate Fe absorption by either

retaining or allowing transcellular transport of intracellular Fe (Mattia et al., 1986). A possible explanation for the interaction between Fe and the ZnAAC is that before or during the occurrence of Zn absorption, Fe successfully competes for the amino acid ligands of ZnAAC with Zn. Apparently, transcellular transport of Fe bound to the amino acid ligands of ZnAAC is not allowed which may have led to increased Fe contents in the small intestine.

Implications

Our findings show that only depleted pigs fed 45 ppm supplemental Zn effectively replenished body Zn stores and regained their ability to grow. Considering the purity of the experimental diet, our data suggest that dietary Zn requirements of weanling pigs are at least 60 ppm Zn. Based on several indicators for assessing Zn status, we can conclude that the ZnAAC did not supply more available Zn than $ZnSO_4$, at least not when included in a semipurified diet fed to Zn depleted or Zn adequate pigs.

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CHAPTER VI

General Discussion

Depleting Porcine Body Pools of Zn. Absorption and excretion processes of Zn are regulated by the animal in order to maintain Zn homeostasis (Aggett, 1991). Therefore, the capacity of Zn absorption increases dramatically when the animal has a high body need for Zn (Hallmans et al., 1987; Menard and Cousins, 1983). The use of highly sensitive animals, viz. Zn depleted pigs, was of importance in our study, since potential differences in availability of Zn between $ZnSO_4$ and Zn amino acid chelate (ZnAAC) might not be found in Zn adequate animals.

A semipurified diet containing 17 ppm Zn was necessary, because 30 ppm Zn provided by a commercial corn soybean meal diet, which is only 30 % below the NRC recommended Zn level for 5 to 10 kg pigs (NRC, 1988), may have been too high to rapidly deplete porcine body Zn stores. Our findings showed that porcine body fluid and tissue Zn stores were sufficiently depleted of Zn within a 3 to 4 wk period. As discussed in Chapter III, growth retardation of Zn deficient pigs seems to be associated with reduced serum mitogenic activity and depressed levels of pituitary RNA. Furthermore, a specific reduction in growth hormone (GH) mRNA was indicated, but further research is necessary to examine whether the reduction in total serum mitogenic activity observed in our study and the previously found reductions in serum insulin-like growth factor-1 levels resulted from a specific depression of GH secretion.

Absorption of Zn. In Exp. 1, samples of digesta of various gastrointestinal (GI) segments were obtained exactly 2.5 h after offering the meal to the pigs using a

method modified from Asche et al. (1989). A critical and basic assumption of the method is that a steady state condition was achieved within all GI segments at the time of sampling. The steady state was defined by Van der Klis et al. (1990) as the condition in the GI tract in which neither the total amount of Cr nor its concentration in the various segments was dependent on the time of feed intake.

In our study, a steady state was assumed to exist 2.5 h after offering the meal, however, it was not validated by determining luminal Cr levels and concentrations in the segments at various time-points after feeding. Therefore, it may be that the large variability in absorption and digestibility coefficients found in primarily the proximal and medial segments of the small intestine resulted from a lack of steady state. Although the method described by Asche et al. (1989) is acceptable, it seems mandatory to verify the existence of a steady state at the time of sampling.

Other intrinsic factors also may have contributed to variability in apparent mineral absorption within the proximal intestine. Firstly, pancreatic secretions may cause variability in apparent absorption coefficients within proximal and maybe medial segments because they are not continuous. Secondly, flow rates of Cr, Zn, Cu, Fe, and DM may have been different for the pigs fed ZnAAC and $ZnSO_4$, since the moisture content in the feces indicated that digesta were less viscous when ZnAAC was used instead of $ZnSO_4$.

It was argued in Chapter IV that intestinal cell proliferation may be lower in pigs fed ZnAAC due to the lower viscosity of the digesta. Alternatively, the supposed reduction in intestinal cell proliferation may be directly related to the form of Zn. Zinc functions as a second messenger of mitogenic induction, and a dramatic reduction in mitogenesis of baby hamster kidney cells was found when EDTA was added to the medium (Grummt et al., 1986). It may be that mitogenic

activity of mucosal cells within the GI tract was reduced when ZnAAC was supplemented to the diet instead of $ZnSO_4$. However, the focus in this study was not on the influence of ZnAAC on the GI morphology. Therefore, future research in the area of chelates and GI morphology is warranted.

The small intestine was an important site of Zn, Cu, Fe and DM absorption. The Zn content of the intestinal mucosa as well as whole intestine mineral contents were highest at those sites in which mineral uptake occurred. In particular levels of intestine Fe and Zn were extremely high in the proximal and distal segments, respectively. Copper absorption was consistently observed from the stomach. An interesting question is, in what form Cu may be absorbed from the stomach? Thermodynamically, binding of Cu with low molecular weight proteins is highly favored (Williams, 1984). Absorption of Cu in a complexed or chelated form in the stomach would explain the disappearance of amino acids from the porcine stomach reported by Asche et al. (1989). However, their suggestion that the residence time differed between amino acids and the solid phase indicator chromic oxide remains valid.

Retention and Availability of Zn. In the first experiment, three levels of Zn, 5, 15, and 45 ppm, were supplemented to the semipurified diet. A dose response trial was necessary to establish an optimum level of Zn for dietary repletion of porcine tissues and fluids. Since depleted pigs fed the 5 and 15 ppm $ZnSO_4$ and ZnAAC diets were not able to replenish their Zn stores, a supplemental level of 45 ppm Zn was used throughout the remainder of the study.

In all three experiments, Zn depleted pigs fed the 45 ppm $ZnSO_4$ and ZnAAC diets quickly regained their appetite and growth ability. Compensatory gain was observed and the Zn contents of body fluids and tissues increased during

the course of Zn repletion. Primarily in rats but also in pigs, it has been observed that Zn depletion is not uniform among tissues (Crofton et al., 1983; Jackson, 1989). Our findings not only confirm this, but also indicate that the same lack of uniformity may be observed during Zn repletion. In general, tissues which were least affected by Zn depletion were repleted faster than tissues which previously lost substantial amounts of Zn.

It may be argued that the degree of sensitivity of tissues to Zn depletion and repletion is indicative of the presence of readily available Zn stores. Of the tissues examined in this study, the liver was the most sensitive to Zn depletion and repletion, whereas, brain and small intestine tissue showed the least sensitivity. Thus, replenishment especially liver Zn stores should be viewed critically in comparing the availability of Zn sources. In addition to the liver, bone is frequently used in bioassays of Zn (Wedekind et al., 1992), because of its sensitivity to Zn depletion and repletion (Hill et al., 1986).

In all three experiments, body fluid and tissue Zn contents were not clearly affected by the Zn source. Especially in pigs repleted with the 15 ppm Zn diets in Exp. 1 which was below their Zn requirement (Smith et al., 1961), differences in Zn availability between the two Zn sources should have resulted in an improved performance and faster tissue Zn repletion. However, this was not observed. Thus, it seems unlikely that the absorption rate and retention of Zn were higher in pigs fed the diets containing ZnAAC compared with $ZnSO_4$.

The high serum mitogenic activity of adequate pigs fed ZnAAC in Exp. 2 may have been caused specifically by the ZnAAC entity. Whether and how the compound ZnAAC induces the synthesis of growth factors warrants further investigation.

Conclusion. Based on numerous indicators of Zn status and absorption data of Zn, we conclude that the availability of Zn from ZnAAC was not markedly different from the commercially used mineral source $ZnSO_4$. The association between the higher disappearance rates of Zn, Cu, Fe, Cr and DM and intestinal luminal conditions of pigs fed ZnAAC remains to be explored.

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CHAPTER VII

Appendix

APPENDIX

Table 1. Serum Zn concentrations of pigs fed the low Zn depletion diet, and of pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn depletion and repletion in Exp. 1

Serum Zn (μg/dL)	Low Zn	Control/Adequate		SEM
		$ZnSO_4$	ZnAAC	
Depletion[a]				
Day 0	96	103	90	3
7[b]	65	103	98	3
14[b]	35	90	81	2
21[b]	27	73	80	2
28[b]	33	81	84	2
32[b]	31	71	74	2
Repletion[c]				
Day 0[b]	33	71	74	5
3[b]	23	73	72	4
6[b]	27	83	84	5
9[b]	25	91	86	5
12[bd]	29	84	95	4
18		101	103	10
24		92	97	7

[a]Each low Zn mean represents 40 pigs and each control mean four pigs

[b]Low Zn mean differs from $ZnSO_4$ and ZnAAC control means ($P < .001$)

[c]Each low Zn mean and each adequate mean represent four pigs

[d]Pigs fed the low Zn depletion diet were taken off study at 12 d

APPENDIX

Table 2. Serum Zn concentrations of Zn depleted pigs fed the low Zn depletion diet supplemented with 5, 15, and 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn repletion in Exp. 1

		Depleted						
		$ZnSO_4$			ZnAAC			
Serum Zn (μg/dL)		5	15	45	5	15	45	SEM
Day	0[b]	30	28	28	33	31	32	2
	3[cde]	20	24	39	20	24	47	3
	6[de]	20	37	71	23	31	61	5
	9[de]	26	41	64	25	38	60	4
	12[def]	28	29	76	34	36	77	5
	18		38	80		28	70	
	24		40	88		26	78	

[a]Each depleted mean represents four pigs initially with one pig sacrificed on d 3, 6, 12, and 24
[b]15 ppm $ZnSO_4$ depleted mean differs from 15 ppm ZnAAC depleted mean ($P < .10$)
[c]45 ppm $ZnSO_4$ depleted mean differs from 45 ppm ZnAAC depleted mean ($P < .05$)
[d]15 ppm $ZnSO_4$ and ZnAAC depleted means differ from 45 ppm $ZnSO_4$ and ZnAAC depleted means ($P < .001$)
[e]15 ppm and 45 ppm $ZnSO_4$ and ZnAAC depleted means differ from 5 ppm $ZnSO_4$ and ZnAAC depleted means ($P < .001$)
[f]Depleted pigs fed the 5 ppm $ZnSO_4$ and ZnAAC diets were taken off study at 12 d

APPENDIX

Table 3. Serum Zn concentrations of pigs fed the low Zn depletion diet and of pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn depletion and repletion in Exp. 2

	Low Zn/Depleted		Control/Adequate		
Serum Zn (μg/dL)	$ZnSO_4$	ZnAAC	$ZnSO_4$	ZnAAC	SEM
Depletion[a]					
Day 0		56	58	57	2
7[b]		31	59	59	2
14[bc]		21	56	48	1
21[bc]		19	62	55	1
24[b]		18	60	61	2
Repletion[d]					
Day 0[e]	18	18	62	66	2
3[e]	33	31	54	50	3
6[e]	39	39	54	60	3
9[f]	40	38	54	53	3
12[e]	48	43	56	63	4

[a]Each low Zn mean represents 32 pigs and each control mean eight pigs

[b]Low Zn mean differs from $ZnSO_4$ and ZnAAC control means ($P < .001$)

[c]$ZnSO_4$ control mean differs from ZnAAC control mean ($P < .05$)

[d]Each depleted mean represents 16 pigs initially with four pigs sacrificed on d 6. Each adequate mean represents four pigs

[e,f]$ZnSO_4$ and ZnAAC depleted means differ from $ZnSO_4$ and ZnAAC adequate means, respectively, ($P < .01$) and ($P < .05$)

APPENDIX

Table 4. Serum Zn concentrations of pigs fed the low Zn depletion diet, and of pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn depletion and repletion in Exp. 3

		Depleted		
Serum Zn (μg/dL)	Low Zn	$ZnSO_4$	ZnAAC	SEM
Depletion[a]				
Day 0	55			2
21	16			1
24	21			1
Repletion[b]				
Day 0[c]		19	23	1
15		62	59	3

[a]Each low Zn mean represents 16 pigs
[b]Each depleted mean represents six pigs
[c]$ZnSO_4$ depleted mean differs from ZnAAC depleted mean ($P < .10$)

APPENDIX

Table 5. Serum alkaline phosphatase (ALP) activity of pigs fed the low Zn depletion diet, and of pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn depletion and repletion in Exp. 1

ALP (U/liter)	Low Zn	Control/Adequate		SEM
		$ZnSO_4$	ZnAAC	
Depletion[a]				
Day 0	196	208	225	10
7	157	180	188	10
14[b]	57	219	202	4
21[b]	38	187	190	2
28[b]	27	210	206	4
32[b]	29	224	219	4
Repletion[c]				
Day 0[b]	35	224	219	11
3[b]	34	231	210	15
6[b]	21	188	193	13
9[b]	18	204	200	19
12[bd]	15	197	191	12
18		175	182	15
24		190	193	12

[a]Each low Zn mean represents 40 pigs and each control mean four pigs

[b]Low Zn mean differs from $ZnSO_4$ and ZnAAC control means ($P < .001$)

[c]Each low Zn mean and each adequate mean represent four pigs

[d]Pigs fed the low Zn depletion diet were taken off study at 12 d

APPENDIX

Table 6. Serum alkaline phosphatase (ALP) activity of Zn depleted pigs fed the low Zn depletion diet supplemented with 5, 15, and 45 ppm Zn either as ZnSO4 or as Zn amino acid chelate (ZnAAC) during Zn repletion in Exp. 1

	Depleted						
	$ZnSO_4$			ZnAAC			
ALP (U/liter)	5	15	45	5	15	45	SEM
Day 0	26	21	26	25	21	23	4
3[b]	33	35	62	28	35	59	7
6[c]	23	50	118	25	37	105	11
9[c]	18	74	179	19	42	156	9
12[cd]	16	73	174	18	57	190	9
18		86	163		57	166	
24		102	144		58	170	

[a]Each depleted mean represents four pigs initially with one pig sacrificed on d 3, 6, 12, and 24

[b,c]15 ppm $ZnSO_4$ and ZnAAC depleted means differ from 45 ppm $ZnSO_4$ and ZnAAC depleted means, respectively, ($P < .05$) and ($P < .01$)

[d]Depleted pigs fed the 5 ppm $ZnSO_4$ and ZnAAC diets were taken off study at 12 d

APPENDIX

Table 7. Serum alkaline phosphatase (ALP) activity of pigs fed the low Zn depletion diet, and of pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn depletion and repletion in Exp. 2

	Low Zn/Depleted		Control/Adequate		
ALP (U/liter)	$ZnSO_4$	ZnAAC	$ZnSO_4$	ZnAAC	SEM
Depletion[a]					
Day 0[b]	90		116	100	5
7[c]	76		139	138	10
14[c]	37		130	138	3
21[c]	21		113	118	4
24[c]	17		104	95	4
Repletion[d]					
Day 0[e]	18	14	99	89	5
3[e]	38	32	113	106	8
6[e]	69	55	114	107	9
9[f]	86	74	126	116	11
12[g]	101	86	125	127	8

[a]Each low Zn mean represents 32 pigs and each control mean eight pigs

[b,c]Low Zn mean differs from $ZnSO_4$ and ZnAAC control means, respectively, ($P < .05$) and ($P < .001$)

[d]Each depleted mean represents 16 pigs initially with four pigs sacrificed on d 6. Each adequate mean represents four pigs

[e,f,g]$ZnSO_4$ and ZnAAC depleted means differ from $ZnSO_4$ and ZnAAC adequate means, respectively, ($P <. 01$), ($P < .05$) and ($P < .10$)

APPENDIX

Table 8. Serum alkaline phosphatase (ALP) activity of pigs fed the low Zn depletion diet and the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn depletion and repletion in Exp. 3

ALP (U/liter)	Low Zn	Depleted		SEM
		$ZnSO_4$	ZnAAC	
Depletion[a]				
Day 0	226			14
21	66			4
24	70			4
Repletion[b]				
Day 0		66	69	7
15		191	209	15

[a]Each low Zn mean represents 16 pigs
[b]Each depleted mean represents six pigs

APPENDIX

Table 9. Body weight of pigs fed the low Zn depletion diet, and of pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn depletion and repletion in Exp. 1

BW (kg)	Low Zn	Control/Adequate		SEM
		$ZnSO_4$	ZnAAC	
Depletion[a]				
Day 0	7.3	6.8	6.8	.2
7	8.3	7.7	7.9	.2
14	10.1	10.3	10.2	.3
21[b]	11.3	12.8	12.4	.3
28[c]	12.0	14.9	14.4	.4
32[c]	12.2	15.8	15.2	.4
Repletion[d]				
Day 0[c]	12.8	15.8	15.2	.7
3[c]	11.0	17.3	16.7	.4
6[c]	11.3	18.8	18.0	.4
9[c]	11.3	20.3	19.2	.5
12[ce]	10.7	21.7	20.7	.6
18		24.8	23.6	.6
24		28.6	27.3	.8

[a]Each low Zn mean represents 40 pigs and each control mean four pigs

[b,c]Low Zn mean differs from $ZnSO_4$ and ZnAAC control means, respectively, ($P < .05$) and ($P < .001$)

[d]Each low Zn mean and each adequate mean represent four pigs

[e]Depleted pigs fed the 5 ppm $ZnSO_4$ and ZnAAC diets were taken off study at 12 d

APPENDIX

Table 10. Body weight of pigs fed the low Zn depletion diet supplemented with 5, 15, and 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn repletion in Exp. 1

	Depleted						
	$ZnSO_4$			ZnAAC			
BW (kg)	5	15	45	5	15	45	SEM
Day 0	13.7	12.4	13.3	12.1	11.1	11.9	.9
3	13.8	12.6	13.7	12.2	11.4	12.1	.7
6	14.6	14.2	15.3	12.9	12.1	13.3	.9
9[bc]	15.2	14.7	16.3	13.8	12.2	13.1	.8
12[cdef]	13.7	16.0	17.9	13.0	13.0	14.3	.8
18		19.2	20.7		16.5	16.4	
24		22.4	24.2		19.3	19.5	

[a]Each depleted mean represents four pigs initially with one pig sacrificed on d 3, 6, 12, and 24

[b,d]15 ppm depleted $ZnSO_4$ mean differs from 15 ppm depleted ZnAAC mean, respectively, ($P < .10$) and ($P < .05$)

[c]45 ppm depleted $ZnSO_4$ mean differs from 45 ppm depleted ZnAAC mean ($P < .05$)

[e]15 ppm depleted $ZnSO_4$ and ZnAAC means differ from 45 ppm $ZnSO_4$ and ZnAAC means ($P < .10$)

[f]Depleted pigs fed the 5 ppm $ZnSO_4$ and ZnAAC diets were taken off study at 12 d

APPENDIX

Table 11. Body weight of pigs fed the low Zn depletion diet, and of pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn depletion and repletion in Exp. 2

	Low Zn/Depleted		Control/Adequate		
BW (kg)	$ZnSO_4$	ZnAAC	$ZnSO_4$	ZnAAC	SEM
Depletion[a]					
Day 0	6.1		6.4	6.2	.3
7	7.2		7.6	7.5	.4
14	8.4		9.1	9.1	.3
21[b]	9.4		10.9	10.9	.4
24[b]	9.5		11.3	11.3	.4
Repletion[c]					
Day 0[d]	9.3	9.8	11.3	11.8	.4
3[d]	9.6	10.2	12.2	12.7	.4
6[d]	10.5	11.1	13.3	13.9	.5
9[d]	11.7	12.3	14.4	15.0	.4
12[d]	12.6	13.1	15.6	16.2	.6

[a]Each low Zn mean represents 32 pigs and each control mean eight pigs

[b]Low Zn mean differs from $ZnSO_4$ and ZnAAC control means ($P < .01$)

[c]Each depleted mean represents 16 pigs initially with four pigs sacrificed on d 6. Each adequate mean represents four pigs

[d]$ZnSO_4$ and ZnAAC depleted means differ from $ZnSO_4$ and ZnAAC adequate means ($P < .01$)

APPENDIX

Table 12. Body weight of pigs fed the low Zn depletion diet, and of depleted pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn depletion and repletion in Exp. 3

		Depleted		
BW (kg)	Low Zn	$ZnSO_4$	ZnAAC	SEM
Depletion[a]				
Day 0	6.9			.5
21	10.5			1.0
24	10.6			1.0
Repletion[b]				
Day 0		10.1	9.9	.8
7		11.8	11.6	.9
14		14.2	13.8	1.1
15		14.7	14.3	1.1

[a]Each low Zn mean represents 16 pigs
[b]Each depleted mean represents six pigs

APPENDIX

Table 13. Performance of pigs fed the low Zn depletion diet, and of pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn depletion in Exp. 1

Item	Low Zn	Control[a]		SEM
		$ZnSO_4$	ZnAAC	
Days 1 - 7				
ADG, kg	.13	.12	.15	.01
ADFI, kg	.33	.27	.31	.02
Gain:feed	.42	.43	.49	.04
Days 8 - 14				
ADG, kg[b]	.25	.38	.33	.02
ADFI, kg	.64	.64	.63	.04
Gain:feed[c]	.40	.60	.53	.02
Days 15 - 32				
ADG, kg[b]	.12	.30	.28	.02
ADFI, kg	.47	.48	.48	.02
Gain:feed[c]	.25	.63	.59	.02
Days 1 - 32				
ADG (kg)[b]	.15	.28	.26	.02
ADFI (kg)	.47	.47	.47	.02
Gain:feed[b]	.32	.59	.57	.02

[a]Each low Zn mean represents 10 pens (four pigs per pen) and each control mean 1 pen (four pigs per pen)

[b,c]Low Zn mean differs from $ZnSO_4$ and ZnAAC control means, respectively, ($P < .01$) and ($P < .05$)

APPENDIX

Table 14. Performance of pigs fed the low Zn depletion diet, and of pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn repletion in Exp. 1

Item		Low Zn	Adequate		SEM
			$ZnSO_4$	ZnAAC	
D 1 - 3	ADG, kg[b]	-.07	.49	.48	.06
	ADFI, kg[b]	.20	.65	.64	.03
	Gain:feed	NE[c]	.75	.76	.22
D 4 - 6	ADG, kg[b]	.09	.51	.45	.05
	ADFI, kg[b]	.37	.71	.69	.03
	Gain:feed	.22	.72	.65	.10
D 7 - 9	ADG, kg[b]	.00	.49	.40	.06
	ADFI, kg[b]	.39	.76	.73	.04
	Gain:feed	NE[c]	.65	.55	.12
D 10 - 12[d]	ADG, kg[b]	-.18	.48	.49	.06
	ADFI, kg[b]	.30	.80	.77	.04
	Gain:feed	NE[c]	.60	.64	.23
D 13 - 18	ADG, kg		.52	.48	.03
	ADFI, kg		.84	.81	.01
	Gain:feed		.62	.59	.03
D 19 - 24	ADG, kg		.63	.62	.03
	ADFI, kg		.93	.90	.02
	Gain:feed		.68	.69	.03
D 1 - 24	ADG, kg		.53	.50	.03
	ADFI, kg		.81	.78	.01
	Gain:feed		.66	.64	.08

[a]Each low Zn mean and each adequate mean represent four pigs
[b]Low Zn mean differs from $ZnSO_4$ and ZnAAC adequate means ($P < .01$)
[c]Not Estimated
[d]Pigs fed the low Zn depletion diet were taken off study at 12 d

APPENDIX

Table 15. Performance of depleted pigs fed the low Zn depletion diet supplemented with 5, 15, and 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn repletion in Exp. 1

		Depleted[a]						
		$ZnSO_4$			ZnAAC			
Item		5	15	45	5	15	45	SEM
D 1 - 3	ADG, kg	.03	.09	.15	.04	.09	.08	.05
	ADFI, kg	.28	.34	.30	.28	.30	.32	.04
	Gain:feed	.13	.21	.49	.17	.33	.22	.16
D 4 - 6	ADG, kg[b]	.16	.47	.41	.04	.32	.43	.06
	ADFI, kg	.43	.48	.53	.36	.40	.42	.06
	Gain:feed	.40	.99	.77	.10	.80	1.03	.11
D 7 - 9	ADG, kg	-.05	.44	.49	-.02	.23	.45	.11
	ADFI, kg	.36	.57	.63	.24	.40	.53	.08
	Gain:feed	NE[c]	.77	.78	NE[c]	.48	.80	.25
D 10 - 12[d]	ADG, kg	-.49	.44	.52	-.26	.25	.42	.13
	ADFI, kg	.26	.63	.68	.13	.48	.58	.07
	Gain:feed	NE[c]	.70	.77	NE[c]	.50	.73	1.29
D 13 - 18	ADG, kg		.41	.46		.36	.34	
	ADFI, kg		.69	.73		.62	.62	
	Gain:feed		.59	.63		.57	.55	
D 19 - 24	ADG, kg		.53	.58		.46	.51	
	ADFI, kg		.77	.81		.69	.68	
	Gain:feed		.69	.72		.67	.75	
D 1 - 24	ADG, kg	.01	.26	.32	.00	.21	.31	.07
	ADFI, kg	.33	.45	.46	.25	.37	.44	.06
	Gain:feed	.08	.52	.68	.04	.55	.71	.15

[a]Each depleted mean represents four pigs initially with one pig sacrificed on d 3, 6, 12, and 24
[b]15 ppm $ZnSO_4$ depleted mean differs from 15 ppm ZnAAC depleted mean ($P < .10$)
[c]Not Estimated
[d]Depleted pigs fed the 5 ppm $ZnSO_4$ and ZnAAC diets were taken off study at 12 d

APPENDIX

Table 16. Performance of pigs fed the low Zn depletion diet, and of pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn depletion in Exp. 2

		Control		
	Low Zn[a]	$ZnSO_4$[a]	ZnAAC[a]	SEM
Days 1 - 7				
ADG, kg	.16	.17	.17	.04
ADFI, kg	.51	.50	.51	.03
Gain:feed	.30	.33	.34	.07
Days 8 - 14				
ADG, kg	.18	.21	.24	.04
ADFI, kg[c]	.54	.58	.57	.01
Gain:feed	.34	.37	.42	.07
Days 15 - 24				
ADG, kg[b]	.10	.22	.21	.01
ADFI, kg[d]	.38	.42	.43	.01
Gain:feed[b]	.27	.53	.50	.03
Days 1 - 24				
ADG, kg[b]	.14	.20	.21	.01
ADFI, kg	.47	.49	.49	.01
Gain:feed[b]	.27	.53	.50	.03

[a]Each low Zn mean represents 8 pens (four pigs per pen) and each control mean 2 pens (four pigs per pen)

[b,c,d]Low Zn mean differs from $ZnSO_4$ and ZnAAC control means, respectively, ($P < .01$), ($P < .10$) and ($P < .05$)

APPENDIX

Table 17. Performance of Zn depleted and Zn adequate pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn repletion in Exp. 2

		Depleted[a]		Adequate[a]		
		$ZnSO_4$	ZnAAC	$ZnSO_4$	ZnAAC	SEM
D 1 - 3	ADG, kg[b]	.10	.13	.30	.29	.04
	ADFI, kg[b]	.26	.29	.41	.41	.02
	Gain:feed	.35	.39	.74	.71	.14
D 4 - 6	ADG, kg[c]	.31	.31	.36	.40	.03
	ADFI, kg[b]	.41	.44	.55	.56	.02
	Gain:feed	.75	.71	.65	.70	.05
D 7 - 9	ADG, kg	.26	.31	.36	.38	.05
	ADFI, kg[b]	.47	.50	.59	.60	.02
	Gain:feed	.57	.61	.62	.63	.09
D 10 - 12	ADG, kg[c]	.29	.26	.40	.40	.04
	ADFI, kg[b]	.51	.55	.62	.64	.02
	Gain:feed	.56	.48	.65	.61	.07
D 1 - 12	ADG, kg[b]	.26	.25	.36	.37	.03
	ADFI, kg[b]	.42	.44	.54	.56	.02
	Gain:feed	.60	.56	.66	.66	.04

[a]Each depleted mean represents 16 pigs initially with four pigs sacrificed on d 6. Each adequate mean represents four pigs

[b,c]$ZnSO_4$ and ZnAAC depleted means differ from $ZnSO_4$ and ZnAAC adequate means, respectively, ($P < .05$) and ($P < .10$)

Appendix

Table 18. Tissue weight, expressed as a percentage of BW, of Zn depleted pigs fed the low Zn depletion diet supplemented with 5, 15, and 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn repletion in Exp. 1[a]

	Depleted					
	$ZnSO_4$[b]			ZnAAC[b]		
Tissue	5	15	45	5	15	45
Day 3						
Liver	2.7	2.8	2.6	2.7	2.6	4.2
Kidney	.26	.28	.29	.31	.28	.33
Day 6						
Liver	2.6	2.8	2.8	3.3	3.0	2.9
Kidney	.24	.25	.24	.29	.26	.20
Day 12[c]						
Liver	2.6	2.8	2.6	2.4	2.2	2.1
Kidney	.28	.21	.22	.27	.29	.22
Day 24[d]						
Liver		2.3	2.2		2.7	2.7
Kidney		.19	.19		.23	.24

[a]Liver and kidney weights of six depleted pigs sacrificed after 32 d of Zn depletion, or d 0 of Zn repletion, were, respectively, 2.86 $\pm$ .33 % and .27 $\pm$ .03 %

[b]Each depleted value represents 1 pig

[c]Depleted pigs fed the 5 ppm $ZnSO_4$ and ZnAAC diets were taken off study at d 12

[d]Liver and kidney weights of the Zn adequate pigs at the end of the 24-d Zn repletion period were, respectively, 2.17 $\pm$ .19 % and .18 $\pm$ .01 % for the four pigs fed the $ZnSO_4$ diet, and 2.26 $\pm$ .19 % and .17 $\pm$ .01 % for the four pigs fed the ZnAAC diet

Appendix

Table 19. Tissue weight of Zn depleted pigs fed the low Zn depletion diet supplemented with 5, 15, and 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn repletion in Exp. 1[a]

	Depleted					
	$ZnSO_4$[b]			ZnAAC[b]		
Tissue, g	5	15	45	5	15	45
Day 3						
Liver	338	288	328	286	306	510
Kidney	33	34	37	32	32	40
Day 6						
Liver	149	319	455	365	403	476
Kidney	31	39	39	32	35	33
Day 12[c]						
Liver	366	417	459	307	260	303
Kidney	37	32	39	28	34	31
Day 24[d]						
Liver		518	527		515	528
Kidney		42	46		44	45

[a]Liver and kidney weights (± SD) of six depleted pigs sacrificed after 32 d of Zn depletion, or d 0 of Zn repletion, were, respectively, 342 ± 93 g and 34 ± 10 g

[b]Each depleted value represents 1 pig

[c]Depleted pigs fed the 5 ppm $ZnSO_4$ and ZnAAC diets were taken off study at d 12

[d]Liver and kidney weights (± SD) of the Zn adequate pigs at the end of the 24-d Zn repletion period were, respectively, 621 ± 79 g and 51 ± 2 g for the four pigs fed the $ZnSO_4$ diet, and 615 ± 36 g and 47 ± 6 g for the four pigs fed the ZnAAC diet

APPENDIX

Table 20. Tissue wet weights of pigs fed the low Zn depletion diet, and of pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) after 24 d of Zn depletion (d 0 of repletion) and after 6 and 12 d of Zn repletion in Exp. 2

Tissue (g)		Low Zn/Depleted		Control/Adequate		
		$ZnSO_4$	ZnAAC	$ZnSO_4$	ZnAAC	SEM
Day 0[a]	Liver	219		279	234	29.4
	Kidney	22		26	24	1.7
	Pancreas[b]	11		16	15	1.2
	Brain	50		51	51	1.4
	Pituitary	.75		.88	.86	.006
	BW, kg[c]	9.3		11.3	9.7	.6
Day 6[d]	Liver[e]	245	296			15.7
	Kidney	26	24			3.0
	Pancreas	15	24			3.4
	Brain	50	49			1.2
	Pituitary[e]	.81	1.0			.006
	BW, kg	9.8	10.5			.7
Day 12[f]	Liver[g]	344	377	402	426	25.9
	Kidney	32	34	35	36	2.8
	Pancreas	26	27	28	32	2.1
	Brain[h]	53	50	56	55	1.5
	Pituitary	1.0	.98	1.1	.99	.005
	BW, kg[i]	12.6	13.1	15.6	16.2	.6

[a]Each low Zn mean represents eight pigs and each control mean four pigs

[b,c]Low Zn mean differs from $ZnSO_4$ and ZnAAC control means, respectively, ($P < .01$) and ($P < .05$)

[d]Each depleted mean represents four pigs

[e]$ZnSO_4$ depleted mean differs from ZnAAC depleted mean ($P < .10$)

[f]Each depleted mean represents eight pigs and each adequate mean four pigs

[g,h,i]$ZnSO_4$ and ZnAAC depleted means differ from $ZnSO_4$ and ZnAAC adequate means, respectively, ($P < .10$), ($P < .05$) and ($P < .01$)

APPENDIX

Table 21. Tissue wet weight of pigs fed the low Zn depletion diet, and of depleted pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn depletion and repletion in Exp. 3

		Depleted		
Tissue, g	Low Zn	$ZnSO_4$	ZnAAC	SEM
Depletion[a]				
Day 24				
Liver	308			29.5
Kidney	27			2.8
BW, kg	12.3			1.0
Repletion[b]				
Day 15				
Liver		328	331	23.2
Kidney		31	30	2.5
BW, kg		14.7	14.3	1.1

[a]Each low Zn mean represents 16 pigs
[b]Each depleted mean represents six pigs

APPENDIX

Table 22. Zinc, Cu, and Fe contents (ppm) in liver, expressed on a DM basis, of pigs fed the low Zn depletion diet supplemented with 5, 15, and 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn repletion in Exp. 1[a]

	Depleted[b]					
	$ZnSO_4$			ZnAAC		
	5	15	45	5	15	45
	----------Zinc----------					
Day 3	71	82	88	80	74	118
6	75	69	117	72	83	106
12[c]	93	72	131	93	97	147
24		76	197		75	141
	----------Copper----------					
Day 3	39	93	37	66	46	69
6	98	60	26	125	57	40
12[c]	134	114	61	90	56	40
24		68	37		27	69
	----------Iron----------					
Day 3	280	177	190	400	149	103
6	139	71	178	148	281	174
12[c]	182	118	141	174	88	470
24		112	64		81	312

[a]Zinc, Cu, and Fe contents (±SD) in livers of six pigs fed the low Zn depletion diet after 32 d of depletion (d 0 of repletion) were, respectively, 70 ± 6, 82 ± 30, and 340 ± 199 ppm.
Zinc, Cu, and Fe contents (±SD) in livers of three pigs fed the 45 ppm $ZnSO_4$ diet during Zn depletion and repletion were, respectively, 245 ± 68, 54 ± 6, and 206 ± 103 ppm.
Zinc, Cu, and Fe contents (±SD) in livers of four pigs fed the 45 ppm ZnAAC diet during Zn depletion and repletion were, respectively, 199 ± 48, 56 ± 7, and 246 ± 151 ppm
[b]Each depleted value represents one pig
[c]Both groups of depleted pigs fed the 5 ppm Zn diets were taken off study at 12 d

APPENDIX

Table 23. Zinc, Cu, and Fe contents (ppm) in kidney, expressed on a DM basis, of pigs fed the low Zn depletion diet supplemented with 5, 15, and 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn repletion in Exp. 1[a]

	Depleted[b]					
	$ZnSO_4$			ZnAAC		
	5	15	45	5	15	45
	----------Zinc----------					
Day 3	91	90	92	85	94	96
6	90	101	96	86	89	98
12[c]	81	93	113	88	88	120
24		82	108		86	105
	----------Copper----------					
Day 3	14	16	17	13	15	27
6	15	20	18	14	19	18
12[c]	15	16	30	16	22	44
24		12	38		15	36
	----------Iron----------					
Day 3	222	250	219	294	235	241
6	161	177	219	214	283	256
12[c]	283	199	218	194	190	275
24		155	169		181	200

[a]Zinc, Cu, and Fe contents (±SD) in kidneys of six pigs fed the low Zn depletion diet after 32 d of depletion (d 0 of repletion) were, respectively, 87 ± 3, 17 ± 2, and 278 ± 46 ppm.
Zinc, Cu, and Fe contents (±SD) in kidneys of four pigs fed the 45 ppm $ZnSO_4$ diet during Zn depletion and repletion were, respectively, 114 ± 9, 26 ± 8, and 163 ± 32 ppm.
Zinc, Cu, and Fe contents (±SD) in kidneys of four pigs fed the 45 ppm ZnAAC diet during Zn depletion and repletion were, respectively, 113 ± 7, 28 ± 8, and 191 ± 73 ppm
[b]Each depleted value represents one pig
[c]Both groups of depleted pigs fed the 5 ppm Zn diets were taken off study at 12 d

APPENDIX

Table 24. Zinc, Cu, and Fe contents (ppm) in proximal small intestinal mucosa, expressed on a DM basis, of pigs fed the low Zn depletion diet supplemented with 5, 15, and 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn repletion in Exp. 1[a]

	Depleted[b]					
	$ZnSO_4$			ZnAAC		
	5	15	45	5	15	45
	----------Zinc----------					
Day 3	83	76	97	80	80	92
6	87	88	93	102	89	105
12[c]	83	88	88	89	87	85
24		88	94		92	89
	----------Copper----------					
Day 3	17	22	10	13	13	15
6	21	13	11	31	14	12
12[c]	12	13	11	12	13	10
24		13	12		12	10
	----------Iron----------					
Day 3	143	112	178	117	177	207
6	117	98	141	162	106	151
12[c]	90	123	93	158	72	193
24		153	137		139	284

[a]Zinc, Cu, and Fe contents (±SD) in mucosa of six pigs fed the low Zn depletion diet after 32 d of depletion (d 0 of repletion) were, respectively, 78 ± 5, 15 ± 3, and 213 ± 75 ppm.
Zinc, Cu, and Fe contents in mucosa of one pig fed the 45 ppm $ZnSO_4$ diet during Zn depletion and repletion were, respectively, 93, 10, and 147 ppm.
Zinc, Cu, and Fe contents in mucosa of one pig fed the 45 ppm ZnAAC diet during Zn depletion and repletion were, respectively, 99, 12, and 296 ppm

[b]Each depleted value represents one pig

[c]Both groups of depleted pigs fed the 5 ppm Zn diets were taken off study at 12 d

APPENDIX

Table 25. Zinc, Cu, and Fe contents (ppm) in medial small intestinal mucosa, expressed on a DM basis, of pigs fed the low Zn depletion diet supplemented with 5, 15, and 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn repletion in Exp. 1[a]

	Depleted[b]					
	$ZnSO_4$			ZnAAC		
	5	15	45	5	15	45
	----------Zinc----------					
Day 3	89	90	103	86	91	96
6	93	104	98	92	87	96
12[c]	88	91	98	81	87	98
24		86	96		84	130
	----------Copper----------					
Day 3	14	18	16	13	13	17
6	13	15	14	23	14	12
12[c]	13	14	11	10	12	17
24		11	18		13	12
	----------Iron----------					
Day 3	94	90	70	107	63	79
6	69	79	78	88	74	97
12[c]	65	73	86	113	59	106
24		75	62		94	87

[a]Zinc, Cu, and Fe contents (±SD) in mucosa of six pigs fed the low Zn depletion diet after 32 d of depletion (d 0 of repletion) were, respectively, 88 ± 7, 14 ± 2 and, 114 ± 17 ppm.
Zinc, Cu, and Fe contents in mucosa of one pig fed the 45 ppm $ZnSO_4$ diet during Zn depletion and repletion were, respectively, 94, 12, and 87 ppm.
Zinc, Cu, and Fe contents in mucosa of one pig fed the 45 ppm ZnAAC diet during Zn depletion and repletion were, respectively, 98, 15, and 111 ppm

[b]Each depleted value represents one pig

[c]Both groups of depleted pigs fed the 5 ppm Zn diets were taken off study at 12 d

APPENDIX

Table 26. Zinc, Cu, and Fe contents (ppm) in distal small intestinal mucosa, expressed on a DM basis, of pigs fed the low Zn depletion diet supplemented with 5, 15, and 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn repletion in Exp. 1[a]

		Depleted[b]					
		$ZnSO_4$			ZnAAC		
		5	15	45	5	15	45
		----------Zinc----------					
Day	3	99	95	105	98	98	102
	6	95	109	117	124	111	107
	12[c]	100	101	117	91	102	117
	24		102	113		93	108
		----------Copper----------					
Day	3	9	9	11	8	9	13
	6	11	14	12	20	12	12
	12[c]	8	11	11	9	9	18
	24		11	10		10	12
		----------Iron----------					
Day	3	105	86	89	129	77	100
	6	76	93	100	99	88	99
	12[c]	83	84	88	140	69	117
	24		90	86		80	109

[a]Zinc, Cu, and Fe contents (±SD) in mucosa of six pigs fed the low Zn depletion diet after 32 d of depletion (d 0 of repletion) were, respectively, 91 ± 4, 11 ± .6, and 127 ± 23 ppm.
Zinc, Cu, and Fe contents in mucosa of one pig fed the 45 ppm $ZnSO_4$ diet during Zn depletion and repletion were, respectively, 92, 8, and 87 ppm.
Zinc, Cu, and Fe contents in mucosa of one pig fed the 45 ppm ZnAAC diet during Zn depletion and repletion were, respectively, 103, 9, and 131 ppm
[b]Each depleted value represents one pig
[c]Both groups of depleted pigs fed the 5 ppm Zn diets were taken off study at 12 d

APPENDIX

Table 27. Serum mitogenic activity (absorbance units) of pigs fed the low Zn depletion diet, and of pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn depletion and repletion in Exp. 2

	Low Zn/Depleted		Control/Adequate		
	$ZnSO_4$	ZnAAC	$ZnSO_4$	ZnAAC	SEM
Depletion[a]					
Day 0	.201		.220	.175	.017
24[b]	.123		.176	.196	.012
Repletion[c]					
Day 0[d]	.136	.118	.176	.196	.012
6[ef]	.210	.234	.228	.312	.021
12	.233	.242	.254	.278	.022

[a]Each low Zn mean represents 32 pigs and each control mean represents eight pigs

[b]Low Zn mean differs from $ZnSO_4$ and ZnAAC control means ($P < .001$)

[c]Each depleted mean represents 16 pigs with four pigs sacrificed on d 6. Each adequate mean represents four pigs

[d,e]$ZnSO_4$ and ZnAAC depleted means differ from $ZnSO_4$ and ZnAAC adequate means, respectively, ($P < .01$) and ($P < .05$)

[f]$ZnSO_4$ adequate mean differs from ZnAAC adequate mean ($P < .05$)

APPENDIX

Table 28. Serum protein concentration (g/dL) of pigs fed the low Zn depletion diet, and of pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) during Zn depletion and repletion in Exp. 2

	Low Zn/Depleted		Control/Adequate		
	$ZnSO_4$	ZnAAC	$ZnSO_4$	ZnAAC	SEM
Depletion[a]					
Day 0	4.22		4.31	4.28	.08
24[b]	4.25		3.99	4.27	.07
Repletion[c]					
Day 0	4.12	4.31	4.07	4.36	.10
6	4.05	4.08	3.97	3.79	.13
12	4.16	4.09	4.25	4.09	.15

[a]Each low Zn mean represents 32 pigs and each control mean represents eight pigs

[b]$ZnSO_4$ control mean differs from ZnAAC control mean ($P < .10$)

[c]Each depleted mean represents 16 pigs with four pigs sacrificed on d 6. Each adequate mean represents four pigs

APPENDIX

Table 29. Pituitary RNA content (μg) and growth hormone mRNA content, expressed relative to $ZnSO_4$ control = 100 %, of pigs fed the low Zn depletion diet, and of pigs fed the low Zn depletion diet supplemented with 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) at the end of Zn depletion in Exp. 2

	Low Zn[a]		Control[a]		
	$ZnSO_4$	ZnAAC	$ZnSO_4$	ZnAAC	SEM
	---------Pituitary RNA----------				
Day 24[b]	2.37		6.74	6.74	.88
	------Growth hormone mRNA-----				
Day 24[c]	36		100	89	19

[a]Each low Zn mean represents 8 pigs and each control mean represents four pigs

[b,c]Low Zn mean differs from $ZnSO_4$ and ZnAAC control means, respectively, ($P < .05$), and ($P < .10$)

APPENDIX

Table 30. Percent apparent absorption of Zn from different gastrointestinal (GI) segments of pigs fed the low Zn depletion diet, and of depleted pigs fed the low Zn depletion diet supplemented with 5, 15, and 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) in Exp. 1[a]

		Depleted[b]						
		$ZnSO_4$			ZnAAC			
GI-segment[c]	Low Zn	5	15	45	5	15	45	SEM[d]
Stomach	- 5.9	- 4.1	-20.2	-18.9	47.8	20.4	20.1	6.6
Proximal Intestine	-23.5	-30.8	-29.6	-56.7	49.4	- 9.5	- 1.3	18.7
Medial Intestine	17.5	5.9	- 3.6	-23.1	28.9	-42.0	27.3	14.1
Distal Intestine	38.2	- 7.8	21.0	15.3	2.8	40.6	38.7	5.8
Cecum	29.5	- 4.7	14.8	14.6	27.2	37.5	41.8	4.7
Proximal Colon	22.5	5.5	10.3	15.8	38.8	31.2	31.7	4.9
Distal Colon	24.1	1.4	15.1	24.3	8.9	37.0	39.1	6.2

[a]Percent apparent absorption of one adequate pig fed $ZnSO_4$ and ZnAAC were, respectively, -.2 and 11.3 % for stomach, -20.5 and -9.0 % for proximal intestine, 15.2 and 4.0 % for medial intestine, 37.0 and 36.0 % for distal intestine, -4.5 and 29.4 % for cecum, 8.9 and 26.2 % for proximal colon, and 8.4 and 42.0 % for distal colon

[b]Each low Zn mean represents six pigs. Each depleted mean represents three (5 ppm) or four pigs (15 and 45 ppm)

[c]See text for definition of each segment

Table 30 (continued)

[d]P-values:

Contrast	Stomach	Small Intestine			Colon		
		Proximal	Medial	Distal	Cecum	Proximal	Distal
Low Zn vs $ZnSO_4$	.486	.579	.210	.0019	.001	.029	.061
Low Zn vs ZnAAC	.0003	.128	.396	.173	.384	.088	.460
$ZnSO_4$ vs ZnAAC:							
1. all values	.0001	.031	.668	.029	.0001	.0002	.006
2. 45 ppm values	.0005	.058	.018	.010	.0005	.035	.092
3. 15 ppm values	.0003	.470	.083	.038	.0026	.007	.011
4. 5 ppm values	.0003	.025	.372	.277	.0004	.0005	.458

APPENDIX

Table 31. Percent apparent absorption of Cu from different gastrointestinal (GI) segments of pigs fed the low Zn depletion diet, and of depleted pigs fed the low Zn depletion diet supplemented with 5, 15, and 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) in Exp.1[a]

		Depleted[b]						
		$ZnSO_4$			ZnAAC			
GI-segment[c]	Low Zn	5	15	45	5	15	45	SEM[d]
Stomach	45.8	36.5	45.6	28.3	56.2	34.6	41.3	7.7
Proximal Intestine	37.4	25.7	36.5	19.7	18.5	36.4	34.2	12.0
Medial Intestine	50.9	38.2	47.4	6.8	- .5	-17.0	41.7	17.8
Distal Intestine	64.9	50.8	47.1	31.3	22.2	47.8	44.2	8.2
Cecum	52.8	18.0	46.6	30.0	24.3	30.4	40.5	4.4
Proximal Colon	50.1	25.1	46.8	26.9	21.2	24.8	35.9	4.5
Distal Colon	54.5	45.3	52.5	32.9	18.8	36.2	50.2	3.0

[a]Each low Zn mean represents six pigs. Each depleted mean represents three (5 ppm) or four pigs (15 and 45 ppm)

[a]Percent apparent absorption of one adequate pig fed $ZnSO_4$ and ZnAAC were, respectively, -.7 and 36.2 % for stomach, 27.1 and 44.2 % for proximal intestine, 18.3 and 40.9 % for medial intestine, 35.8 and 66.8 % for distal intestine, 5.3 and 50.1 % for cecum, 14.5 and 45.5 % for proximal colon, and 19.7 and 54.9 % for distal colon

[c]See text for definition of each segment

Table 31 (continued)

[d]P-values:

Contrast	Stomach	Small Intestine			Colon		
		Proximal	Medial	Distal	Cecum	Proximal	Distal
Low Zn vs $ZnSO_4$	.034	.433	.228	.013	.0001	.0002	.0001
Low Zn vs ZnAAC	.653	.749	.081	.035	.0021	.0010	.0001
$ZnSO_4$ vs ZnAAC:							
1. all values	.063	.602	.512	.618	.015	.423	.380
2. 45 ppm values	.239	.432	.177	.280	.108	.171	.0011
3. 15 ppm values	.319	.999	.027	.951	.018	.002	.0009
4. 5 ppm values	.171	.714	.194	.045	.396	.602	.0001

APPENDIX

Table 32. Percent apparent absorption of Fe from different gastrointestinal (GI) segments of pigs fed the low Zn depletion diet, and of depleted pigs fed the low Zn depletion diet supplemented with 5, 15, and 45 ppm Zn either as $ZnSO_4$ or as Zn amino acid chelate (ZnAAC) in Exp. 1[a]

		Depleted[b]						
		$ZnSO_4$			ZnAAC			
GI-segment[c]	Low Zn	5	15	45	5	15	45	SEM[d]
Stomach	-37.4	-10.4	.3	-25.2	7.1	-15.0	-29.7	11.5
Proximal Intestine	23.4	13.3	26.0	6.1	3.1	-44.2	16.9	20.9
Medial Intestine	49.6	44.9	39.4	40.8	21.7	5.2	33.7	12.0
Distal Intestine	54.4	63.0	38.3	31.8	50.7	47.7	43.9	20.1
Cecum	-44.6	-52.9	-12.3	-23.3	-22.9	-57.1	- 5.2	20.4
Proximal Colon	6.6	-13.8	11.5	14.6	- .8	- 4.4	12.1	9.1
Distal Colon	11.9	37.5	21.1	10.7	7.1	13.6	14.3	5.3

[a]Percent apparent absorption of one adequate pig fed $ZnSO_4$ and ZnAAC were, respectively, -108.8 and -39.7 % for stomach, 23.9 and 47.1 % for proximal intestine, 43.1 and 41.1 % for medial intestine, 36.7 and 37.0 % for distal intestine, -43.7 and 7.2 % for cecum, -1.3 and 12.9 % for proximal colon, and -19.5 and 18.7 % for distal colon

[b]Each low Zn mean represents six pigs. Each depleted mean represents three pigs (5 ppm) or four pigs (15 and 45 ppm)

[c]See text for definition of each segment

Table 32 (continued)

[d]P-values:

Contrast	Stomach	Small Intestine			Colon		
		Proximal	Medial	Distal	Cecum	Proximal	Distal
Low Zn vs $ZnSO_4$	.913	.784	.561	.162	.602	.696	.942
Low Zn vs ZnAAC	.154	.426	.074	.249	.263	.868	.967
$ZnSO_4$ vs ZnAAC:							
1. all values	.153	.566	.167	.755	.500	.804	.898
2. 45 ppm values	.784	.751	.683	.273	.542	.849	.703
3. 15 ppm values	.350	.025	.059	.393	.139	.234	.403
4. 5 ppm values	.445	.762	.255	.383	.383	.395	.014

Appendix

PROCEDURE ANAL-2 SPECTROPHOTOMETRIC ASSAY

Mineral assay of feed, digesta, tissues and serum

Han Swinkels and Wei Zhou

The method used to analyze minerals consists of two parts. Firstly, digestion of samples using a wet ashing procedure (AOAC, 1990). Secondly, determination of the mineral content using Atomic Absorption Spectrophotometry (Perkin Elmer, Model 5100 PC, Serial #: 132658).

In the protocol the cleaning process of digestion tubes, sample preparation of feed, digesta, tissues and serum, and spectrophotometric analysis of the minerals zinc, copper, iron and chromium will be described. The procedure is applicable for analyzing other samples and reading other minerals.

EXPERIMENTAL PROTOCOL

a. Cleaning of Digestion Tubes

1) Wash tube thoroughly with brush and detergent (Liquinox, Alconox, Inc., New York, NY). Rinse with hot water.

2) Boil tubes in water for at least 1 h. After boiling rinse tubes with distilled water.

   Note: Washing and boiling removes organic matter.

3) Rinse tubes with distilled water and soak for at least 4 h in 2.5 % chelate bath (Multi-Terge, Diagnostic Systems, Inc., Gibbstown, NJ). Thereafter, rinse tubes thoroughly with distilled water.

   Note: Chelating agent removes minerals.

4) Incubate tubes for at least 12 h in a 8 N HNO_3 bath. Thereafter, rinse tubes very thoroughly with distilled water.

Note: Minerals are brought into solution in a strong acidic environment.

5) Dry tubes completely in drying oven for glassware. Thereafter, store tubes in sealed plastic bags.

Note 1: **"Clean"** is the key for reliable results!

Note 2: Blanks can be run with each digestion to verify proper cleaning.

b. Wet Ashing of Feed and Digesta

1) Put a weighed aliquot of sample into the digestion tube.

* 0.5 to 1 g is recommended for Feed and Digesta
* 1 to 3 g is recommended for Tissues (wet)
* 0.2 g is recommended for mineral premixes

Note 1: Make sure that all samples are properly mixed and homogeneous before weighing out.

Note 2: The assay should be run in duplicate or triplicate.

2) Add 5 ml of 16 N Nitric Acid (HNO_3) to each of the tubes. Swirl the tube gently during the addition of acid. Thereafter, let the tubes sit for minimal 12 h in an active acid hood.

Note: Wear protective glasses and gloves when working with Nitric Acid.

3) Boil the tubes at moderate temperature on heating block until organic matter is boiled off (brown fumes disappear and solution looks clear).

Note 1: Tubes will boil dry if boiled too long or at high temperature. Check at short intervals.

Note 2: Repeat addition of Nitric Acid (after cooling down sample) if solution is not clear.

4) Take the tubes off the heating block and let them cool down (approximately 15 min). Thereafter, add 3 ml 12 N $HClO_4$. Swirl the tube gently during the addition of acid.

Note: Wear protective glasses and gloves when handling tubes and when working with acid.

5) Boil the tubes at moderate temperature on heating block until dense white fumes appear. Thereafter, take tubes off heating block and let them cool off. Thereafter, add distilled water for diluting sample.

Note: Boiling off perchloric acid is a timely process. Checking at short intervals is less critical.

c. Reading Minerals Using Flame Atomic Absorption Spectrophotometer

1) Standards: Make up series of minimal five standards using the "Standard Reference Solutions".

* For Cu, Cr and Fe analyzed in feed, digesta, and tissues standard series of 1, 2, 3, 4 and 5 ppm were used. For Zn, a standard series of .5, 1, 1.5, 2 and 2.5 ppm was used.

* For serum Zn a standard series of .2, .4, .6, .8 and 1.0 ppm in 10% glycerin is used. Glycerin is added to mimic the viscosity of serum at low dilutions.

Note 1: Store standards in acid washed plastic bottles.

Note 2: It is suggested to use the same standard series for analyses of samples within a experiment.

2) Samples: Dilute the digested sample so its reading falls within the range of the standard curve.
A coefficient of variation of 5% between duplicates or triplicates is acceptable as upper limit.

Note 1: The most accurate readings lie within the mid-section of the standard curve.

Note 2: When reading serum zinc (diluted 1 to 2 with distilled water), it is recommended to aspirate distilled water in between readings.
This will keep the nebulizer clean!

LIST OF CHEMICALS USED IN THE MINERAL ANALYSIS

Company	Catalog #	Compound
FISHER	A200-212	Nitric Acid (HNO_3) Reagent A.C.S. (69 - 71%)
FISHER	A229-8Lb	Perchloric Acid ($HClO_4$) Reagent A.C.S. (70%)
FISHER	SC192-500	Chromium Reference Sol. (1,000 ppm)
FISHER	SO-C-194	Copper Reference Sol. (1,000 ppm)
FISHER	SI124-500	Iron Reference Sol. (1,000 ppm)
FISHER	SZ13-500	Zinc Reference Sol. (1,000 ppm)
FISHER	G31-1	Glycerin (Mr 92.1)

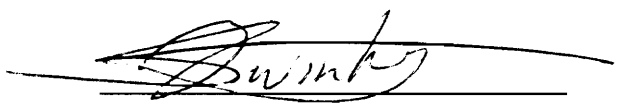
REFERENCE

AOAC. 1990. Official Methods of Analysis. (15th Ed.). Association of Analytical Chemists, Arlington, VA.

VITA

Johannes Wilhelmus Gerardus Maria Swinkels, son of Theodorus Swinkels and Petronella Maria Swinkels-Van den Berg, was born on May 28, 1962 in Milheeze, the Netherlands. In 1981, he graduated from the Sint Willibrordus Gymnasium at Deurne, the Netherlands. Han continued his education at the Department of Animal Science at the Agricultural University of Wageningen, the Netherlands. He graduated in 1988 with a Master's Degree in Animal Science, majoring in Animal Nutrition and Animal Husbandry. He then moved to the United States and attended the College of Agriculture and Life Sciences at Virginia Polytechnic Institute and State University, Blacksburg, VA. He received his Doctorate in Animal Science in 1992.

Han is an active member of several professional societies including the American Society of Animal Science and the American Association for the Advancement of Science.



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