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Review:

Treatment of zinc deficiency without zinc fortification

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Abstract: Zinc (Zn) deficiency in animals became of interest until the 1950s. In this paper, progresses in researches on physiology of Zn deficiency in animals, phytate effect on bioavailability of Zn, and role of phytase in healing Zn deficiency of animals were reviewed. Several studies demonstrated that Zn is recycled via the pancreas; the problem of Zn deficiency was controlled by Zn homeostasis. The endogenous secretion of Zn is considered as an important factor influencing Zn deficiency, and the critical molar ratio is 10. Phytate (inositol hexaphosphate) constituted up to 90% of the organically bound phosphorus in seeds. Great improvement has been made in recent years on isolating and measuring phytate, and its structure is clear. Phytate is considered to reduce Zn bioavailability in animal. Phytase is the enzyme that hydrolyzes phytate and is present in yeast, rye bran, wheat bran, barley, triticale, and many bacteria and fungi. Zinc nutrition and bioavailability can be enhanced by addition of phytase to animal feeds. Therefore, using phytase as supplements, the most prevalent Zn deficiency in animals may be effectively corrected without the mining and smelting of several tons of zinc daily needed to correct this deficiency by fortification worldwide.

Key words: Zinc (Zn), Phytate, Phytase, Zinc deficiency, Zinc homeostasis
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INTRODUCTION

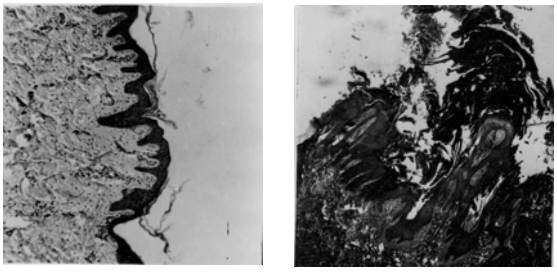
Historically, zinc (Zn) was considered to be required in such small amounts that zinc deficiency was considered to be improbable. Most pigs and chickens were farrowed and grown on pastures and acceptable growth rates were experienced. The animals could stir the topsoil and meat and bone scraps or fishmeal was considered an essential part of diets for pigs and chickens. Thus enough zinc was provided to meet their basic needs. The discovery and successful synthesis of Vitamin B_{12} eliminated the need for the animal protein additions to the diets of monogastric animals. In the early 1950s, animal scientists considered that all of the essential nutrients had been discovered. Farmers, thinking progressively, wanted to move their herds into barns where concrete slab floors were easier to clean thus requiring less labor. It was also a theory at that time that more calcium (Cd) would be needed to provide stronger bones when animals were maintained on concrete slabs. This combination of circumstances quickly declared a disaster because these animals, denied access to soil, failed to grow and developed a skin condition that was described as parakeratosis (Kernkamp and Ferrin, 1953). Shortly thereafter, parakeratosis was described as a zinc deficiency disease with swine fed a corn-peanut protein diet (Tucker and Salmon, 1955). The relationship between calcium and zinc was further extended by Newland *et al.*(1958). The first demonstration that phytate (inositol hexaphosphate) was involved in the onset of zinc deficiency was demonstrated by O′Dell and Savage (1960). At that time general thoughts were that phytate affected zinc absorption or availability, and thus the term bioavailability was coined. As early as 1943, Montgomery *et al.*(1943) had shown that zinc was secreted into the duodenum of the dog. This has been subsequently repeated in seven species including man (Sullivan *et al.*, 1965). It has been quantified to be

equivalent to at least 2~4 times the amount of zinc consumed within each day (Oberleas, 1996). At the same time, it has been determined that only 4% of the phytate is hydrolyzed during passage through the GI (gastro intestimal) tract (Oberleas, 1964). There is a small amount of phytate synthesis by colonic bacteria that can be modified by dietary manipulation. Thus, if a phytase enzyme was available for adding to the diets of monogastric animals and man, zinc deficiency could be treated effectively by reducing the phytate during passage through the GI tract. More of the endogenous zinc would be reabsorbed, thus effectively treating zinc deficiency.

ZINC DEFICIENCY

Zinc deficiency did not become of interest until the 1950s when animal scientists attempted to move swine and chickens from facilities that allowed these animals access to soil. At the same time, there was some concern that the concrete floors would increase the stress on the bones and thus additional calcium may be required to strengthen the bones, particularly of swine. Animal scientists also assumed that a diet of corn (maize) and soybean meal with NaCl, Ca, P, Fe and vitamins would provide an adequate diet. This combination of events resulted in poor growth and in the latent stages pigs developed parakeratosis (Fig.1) (Kernkamp and Ferrin, 1953). It was soon determined that this symptomatology could be prevented with zinc supplementation of a diet of corn and peanut meal as the protein source (Tucker and Salmon, 1955). The primary difference between animal proteins and plant proteins was the presence of phytate in plant seeds. O′Dell and Savage (1960) used the chick to test this theory with the results shown in Table 1, which showed the positive effect of zinc supplementation. These effects were later shown in pigs (Oberleas *et al*., 1962) and rats (Oberleas *et al*., 1966). Many people questioned this because only about 25% of the dietary zinc was absorbed. The outcome was so predictable that a mathematical formula was derived to fit the data using 147 rats. The formula was developed as: phyate:zinc molar ratio= $(m_P/660)/(m_{Zn}/65.4)$, where m_P is the weight of dietary phytate (g), m_{Zn} is the weight of dietary zinc (g), and 660 and 65.4 are molecular weight of undissociated phytic acid and atomic weight of zinc, respectively.

It was later determined that endogenously secreted zinc was an important aspect of the observed phenomenon and that this formula predicted zinc homeostasis rather than absorption. The critical molar ratio was determined by Lo *et al*.(1981) with molar ratios less than 10 representing zinc adequacy and those greater than 10 representing zinc deficiency. The theoretical model for zinc homeostasis is shown in Fig.2.



(a) (b)

Fig.1 Histology of normal pig skin (a) and zinc deficient parakeratotic pig skin (b) (Oberleas, 1964)

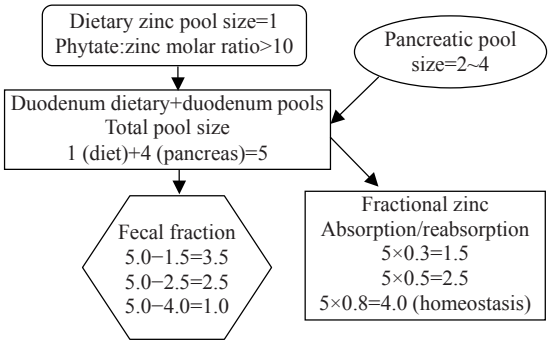


Fig.2 Theoretical homeostasis model for all monogastric animals illustrating the relative pool sizes (Oberleas, 1996)

Table 1 First demonstration of phytate effect on availability of zinc for chick growth*

Trials	Casein-gelatin diet (g)				Soybean protein diet (g)		
	Basal diet	Basal+ 55×10^{-6} Zn	Basal+ phytate	Basal+phytate+ 55×10^{-6} Zn	Basal diet	Basal+15×10^{-6} Zn	Basal+ 55×10^{-6} Zn
1	460	469	206	473	162	382	473
2	447	446	153	395	122	391	440

*Weight (g) after 4 weeks treatment, n=10 (O′Dell and Savage, 1960)

PHYTATE

Phytate (inositol hexaphosphate) (Fig.3) was isolated from aleurone grains from plant seeds in (Pfeffer, 1872). Subsequent studies determined that it constituted up to 90% of the organically bound phosphorus in seeds. The technology available early in the 20th century made it difficult to isolate phytate and study its structures and chemical properties. Thus the first analytical method for phytate was published in (Heubner and Stadler, 1914). This method lacked specificity, accuracy and precision. Several modifications of this original method have been developed based on the basic chemical principles that phytate forms very insoluble ferric iron complexes in dilute acid. From this point, many modifications have been employed by measuring the iron bound, the phosphate complexed, wet-ashing the precipitated complex, dry-ashing the precipitated complex, and a variety of colorimetric measurements of either precipitated iron or phosphate, or differential measurements of iron or phosphate. With the advent of ion exchange and liquid chromatography, methodologies have improved to provide sensitivity, precision and specificity. Still other studies have shown that there was only about 4% net hydrolysis on passage through the GI tract of a rat and there was a small amount of synthesis by colonic bacteria (Oberleas, 1964).

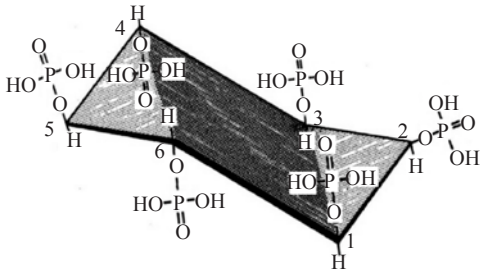


Fig.3 Undissociated phytic acid molecule (Oberleas, 1971)

CHEMISTRY AND PHYSIOLOGY

Several studies have demonstrated that zinc was recycled via the pancreas (Fig.4). The first two demonstrations were done with dogs (Birnstingl *et al*., 1956; Montgomery *et al*., 1943). This was followed with mice (Cotzias *et al*., 1962), pigs (Pekas, 1966), dairy cows and calves (Miller and Cragle, 1965), humans (Sullivan *et al*., 1965) and rats (Oberleas, 1996). Many of these studies were not quantified but some indicated that 2 to 4 times as much zinc was secreted as was consumed from dietary sources. Thus, the problem was zinc homeostasis, because the effect of phytate is on the duodenal pool of zinc (dietary plus pancreatic secretion) rather than dietary absorption alone. Zinc is the only essential cation secreted via the pancreas and is the essential divalent cation that binds tighter to phytate than other essential cations (Fig.5) (Oberleas and Chan, 1997).

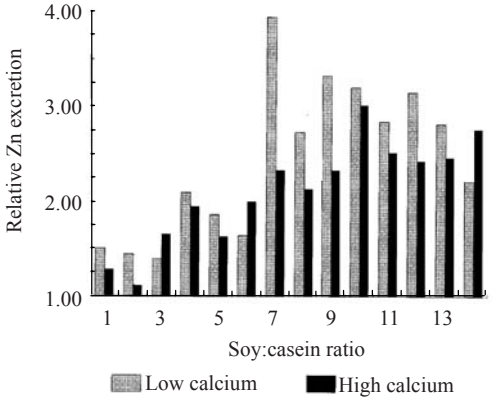


Fig.4 Ratio of zinc excretion from rats injected intraperitonially with radioactive zinc illustrates the relative effect of phytate on fecal secretion of ^{65}zinc. Values greater than 1 represent the effect of phytate on the endogenous pool of zinc (Oberleas, 1996)

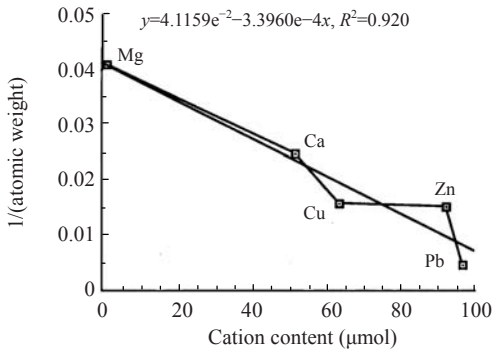


Fig.5 The correlation between atomic weight and the concentration for each cation precipitated at the initial phytate:cation molar ratio of 1:2 at pH 6 in vitro. The correlation R^2=0.92 (Oberleas and Chan, 1997)

In animals, zinc is intimately involved in cell division (Fig.6). Zinc serves as a cofactor in RNA polymerase and reverse transcriptase and in zinc-finger proteins that are adducts to DNA, and

regulates the expression of DNA by serving as receptor sites for hormone and other in vivo factors. In the absence of zinc, the protein moieties may be synthesized but the regulation fails. There are several important inducible enzymes whose synthesis is regulated by zinc-finger proteins, namely thymidine kinase and lactic dehydrogenase that are not adequately expressed during zinc deficiency and thus the synthesis of these enzymes is compromised (Fig.7). Thymidine kinase is the enzyme studied thus far that demonstrates the greatest sensitivity to zinc depletion and thus the synthesis of zinc-finger proteins is the most sensitive metabolic zinc dependent process in the body. It is not clear whether similar zinc-finger proteins are expressed in plants.

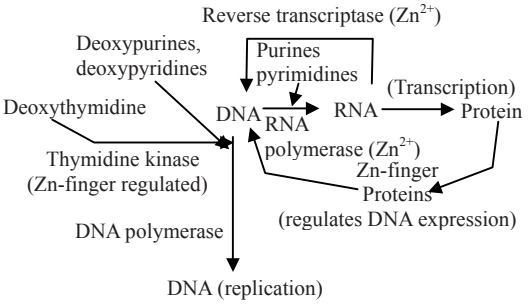


Fig.6 Some of the important zinc functions involved in cell division

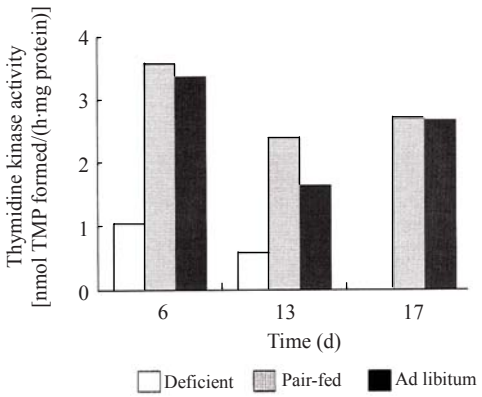


Fig.7 Effect of zinc status and dietary feeding patterns on the relative activity of thymidine kinase activity (Prasad and Oberleas, 1974)

PHYTASE

With this knowledge, two possible solutions arose for the treatment of zinc deficiency; either by adding extra zinc to the diet or by reducing the phytate in the diet prior to or during digestion. With the intensification of swine and chicken management, a new problem arose. Undigested phytate phosphorus was not available for absorption or utilization. Moreover, extra inorganic phosphate was added to the diets to meet the needs of the animals and undigested phytate became a part of the excreted fecal matter. This large amount of fecal phytate was digested by colonic and soil bacteria, and the phosphate became excessive and toxic to plants grown on these soils. Phytase, the enzyme that hydrolyzes phytate, is present in yeast, rye bran, wheat bran, barley, triticale, and many bacteria and fungi. Though more phytate composition data are needed, it has been shown that with phytate:zinc molar ratios of 25, all zinc parameters studied could be counteracted completely by the addition of 1000 phytase units per kg diet when fed to rats or pigs without undesirable side effects. Phytase preparations are available today from the fungus, *Aspergillus ficuum*, a genetically modified variety of *Aspergillus niger*. It has been used as a dietary supplement suitable and effective for this purpose (Fig.8). This is currently the product of choice because it has an effective range of activity from about pH 2.5 to 6 comparable to the stomach and small intestine.

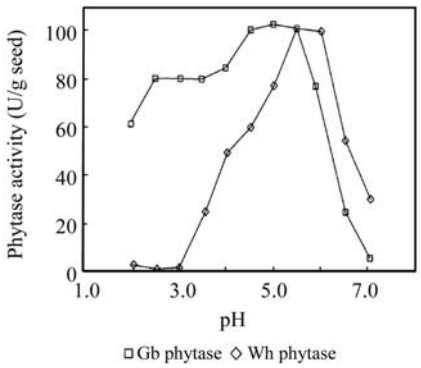


Fig.8 Relative effect of pH on phytase activity in vitro from wheat (Wh) and Gist-brocades (Gb), Delft, the Netherlands microbial phytase (Eeckhout and de Paepe, 1996)

CONCLUSION

Few studies have been done to test the efficacy of treating zinc deficiency in a similar manner. A series of studies conducted with pigs and rats are

summarized (Pallauf and Rimbach, 1996). Increased absorption of phosphate and absorption/reabsorption of zinc was improved, but this concept deserves additional studies with different levels of phytase and different phytate:zinc molar ratios. There is great hope that this most prevalent deficiency may be effectively corrected without the mining and smelting of several tons of zinc daily needed to correct this deficiency by fortification worldwide.

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