A LONG WAYS FROM OLD BLACK MAGIC: TRACE MINERAL ABSORPTION MECHANISMS IN PIGS

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BACKGROUND

In the 1991 **Swine Nutrition** book (edited by Miller, Ullrey and Lewis), J.C. Pekas stated that "it is difficult to distinguish digestion from absorption." While Pekas was referring more to the capacity to absorb and digest and environmental factors, it is true that 20 years ago absorption in the gastrointestinal tract was a black box with magical powers. While in this same text, E. R. Miller never mentioned how absorption occurs for Fe, Cu, Zn, and Mn while today, we talk about valence changes, transport proteins, receptors, binding proteins, regulatory proteins, storage proteins, etc. The Black Box now has Black Magic!

IRON

It has been known for some time and is very important in human nutrition that Fe in animal protein is more available for metabolic functions than Fe bound as non-heme Fe. With the more advanced molecular laboratory techniques, we now know this is because of the "extra steps" for non-heme Fe in feed and food before it can cross the brush border into the entrocyte. The bound non-heme Fe is acted on by HCl and proteases in the stomach with additional action of enzymes in the small intestine to ultimately be either Fe^{2+} or Fe^{3+}. The Fe^{2+} is then bound to the primary transporter of divalent cations, divalent metal transporter (DMT1) in the brush border membrane of the intestinal cell. These are located primarily in the duodenum. The DMT1 transporter is coupled with a hydrogen transport system called symport for the entry of Fe and other cations into the enterocyte. However, Fe^{3+} (ferric) ions movement into the intestinal cell is not as well known. It appears that ferric Fe must bind to a ligand or chelator to help solubilize it for the ultimate binding of the Fe^{3+} molecule to intgrin for absorption across the brush border.

The absorption of non-heme Fe is enhanced by dietary factors that assist in the conversion and maintenance of Fe into ferrous Fe (Fe^{2+}) form. Known to improve Fe absorption are fructose, sorbitol, ascorbic acid, citric acid, lactic acid, mucin, and meat products. Dietary factors known to reduce Fe absorption include oxalic acid, phytate, etc. Altering the ratio of other minerals such as Ca, Zn, Mn and Ni can also reduce Fe absorption. This is probably due to competition for transporter binding sites.

The absorption of Fe is tightly controlled at the intestinal level, and Fe deficient animals absorb an increasing amount of Fe until the Fe status is returned to normal. One of the controls of Fe absorption is hepcidin (HAMP) that is released from the liver when the Fe stores are adequate or high. This protein (hepcidin) causes the internalization of the protein, ferroportin, so that Fe cannot be transported out of the enterocyte.

When Fe is needed by the body, no hepcidin is made so ferroporitin transports Fe (Fe^{2+}) out of the enterocyte. Ultimately the Fe molecule again has a change of valence by ceruloplasmin or hephaestin to Fe^{3+} so it can bind to an apotransferrin and be carried in the blood by transferrin. Both of these valence changing proteins with ferroxidase activity (oxidize ferrous to ferric) are Cu containing proteins. When animals are Cu deficient and perhaps even if only marginally Cu deficient, there will be reduced Fe transport, and Fe will accumulate in the intestine, liver, etc.

The roles of these proteins, interactions with nutrients and relationships within the GI tract will become clearer with our illustrations.

Recent swine Fe research

Spear's laboratory (Hansen et al., 2009) has shown that DMT1 and ZIP14 (solute carrier family 39 family 14), were up-regulated in the intestine when they produced Fe deficiency in young pigs. They also reported a 6.25 fold increase in the hepatic expression of hepcidin in pigs fed a high Fe diet vs. those fed the control diet (Hansen et al., 2010).

Our laboratory (Rincker et al., 2004) reported that when pigs had Fe added to their diet (0, 25, 50, 100, 150 ppm) for 35 d, their hepatic Fe stores were lower than the Fe concentration in the livers of contemporary newly weaned pigs. This may indicate that dietary Fe was not adequate or that the metabolic mechanisms controlling absorption prevented excessive Fe absorption. It is noteworthy that plasma transferrin (Fe transport protein) was lower in pigs fed 50, 100 or 150 ppm Fe. Hepatic Fe concentrations were maximized at 100 ppm Fe in the diet, but the whole body Fe concentration was maximized at 50 ppm of dietary Fe (Rincker et al., 2004). When these same diets were fed in a balance study, the amount retained per day was greatest for pigs fed 150 ppm Fe, but the percent retained did not differ for pigs fed any of the diets (Rincker et al., 2005). The effects of supplemental Fe were found to affect the binding activity of iron regulatory proteins (IRP) that control the posttranscriptional expression of Fe storage and transport proteins (Rincker et al., 2005).

ZINC

Like Fe, the hydrolysis of dietary Zn from amino acids, nucleic acids, etc. has to occur via the digestive processes in the stomach and small intestine for Zn absorption to occur. Primarily in the proximal small intestine, Zn is absorbed into the enterocytes by a carrier-mediated process. The protein carrier is ZIP4 (also called Zrt and Irt-like protein). The exact mechanism is not well understood. While DMT1 mRNA expression is up-regulated by Zn, this Fe transporter appears to transport very little Zn into enterocytes. High concentrations of Zn such as pharmacological concentrations (2,000 to 3,000 ppm) are thought to be absorbed by passive diffusion and paracellular Zn absorption.

The absorption of Zn is thought to be enhanced by substances that are ligands. Usually Zn binds to the S or N in amino acids such as cysteine, histidine, glutamine, glycine. It appears that the binding to ligands helps to maintain the solubility of Zn in the GI tract. It is not clear if Zn bound to amino acids is absorbed via amino acid transporters.

Many components of feed complex with Zn and reduce or inhibit its absorption. The best known is phytate or phytic acid that is especially high in corn. The zinc/phytate complex is formed with the use of oxygen and is very large. The fermentation and/or soaking process will reduce some of the negative effects of this binding. Some fibers are also known to bind Zn and inhibit absorption. Hence, gestation diets where dietary fiber is increased may have reduced Zn availability.

Other divalent cations such as Fe, Ca and Cu interact with Zn by competing for binding ligands in the lumen of the GI tract or within the brush border for transporters. The interaction of Zn and Cu involves a small protein with many S residues, metallothionein (Mt). The synthesis of Mt is stimulated by Zn and Cu, but it preferentially binds Cu over Zn.

There are questions about how Zn is managed in the enterocyte, but intracellular Zn is bound to cysteine-rich intestinal protein (CRIP) or Mt. If the cell is sloughed, the bound Zn is lost in the feces (Rincker et al., 2005b). It is known that this occurs when pharmacological Zn is fed (Carlson et al., 1999). The Zn that is not bound to Mt is transported across the basolateral membrane and is carried by Zn transporters (ZnT). The ZnT- 1 transporter is found in many tissues including the enterocyte basolateral membrane and is known to preferentially move Zn out of the duodenum and jejunum. There are several ZnT and ZIP proteins involved in the influx and efflux of Zn from cells.

Zinc is transported in the blood primarily by albumin where it is loosely bound. It can also be found associated with transferrin, ∂-2 macroglobulin, and immunoglobulin (Ig)G for transport. Histidine and cysteine can transport Zn, also.

COPPER

The concentration of Cu in grow-finish swine diets appears to exceed their needs. However, the requirement for Cu throughout the life cycle is only an estimate. Endogenous Cu secreted by the pancreas, intestine and bile are recycled to maintain Cu status as necessary.

Most Cu in the GI tract is in the Cu^{2+} form and is often bound to amino acids. As with Fe and Zn, the digestion process must free Cu before absorption. The duodenum is the primary site of absorption, but it can be absorbed in the jejunum and perhaps in the ileum. The means by which Cu crosses the brush border is unknown; perhaps being bound to an amino acid in the lumen may assist in its presentation to the brush border receptors. The divalent transporter, DMT1, may also have a role. The most important transporter found in many tissues including the intestine and the liver, the primary storage organ, is Ctr1. It is thought that the Cu ion must be reduced to Cu^{1+} before transport across the brush border. The percent of Cu absorbed has been shown to increase when the Cu in the diet and the body is reduced.

Amino acids, especially those that contain S, assist in enhancing Cu absorption. Like Zn, it is not known if Cu bound to amino acids is absorbed via amino acid transport system(s). Organic acids improve absorption. Like Zn, phytate inhibits the absorption of Cu by forming a complex that is primarily excreted in the feces. As previously noted, Zn and Cu stimulate the synthesis of Mt which preferentially binds Cu over Zn. Feeding pharmacological Zn for extended periods of

time will induce a Cu deficiency by this mechanism (Hill et al., 1983). Large amounts of Fe will also reduce Cu absorption, and this is a concern in diets with excessive Fe is brought into the diet by contaminants of feed ingredients. Tetrathiomolybdate is formed as an insoluble complex when these nutrients (Mo, S, Cu) are not fed in the correct relationship. The risk occurs because this complex can circulate in blood, but cannot be absorbed into the cell.

The management of Cu within the cell has been studied extensively. When Ctr1 presents Cu to the cell, various chaperone proteins carry Cu to specific parts of the cell for specific enzymatic synthesis. Cu chaperone protein for Cu/Zn superoxide dismutase (CCS) is very responsive to the Cu status of the animal and appears to be a possible status indicator. We have recently found this intra-cellular transporter in pigs fed organic Cu. Another intracellular transporter, Ctr2, carries Cu^{1+} to storage vesicles within the cell cytoplasm. Cox 17 in the cytosol and Cox11 in the mitochondria transport Cu^{1+} for cytochrome C oxidase synthesis. While Atox1 brings Cu^{1+} to phosphorylation type of ATPases that are necessary for the export of Cu. There are several other Cu chaperone proteins.

Like Zn, Cu is transported by albumin, a specific Cu transport protein, and histidine and cysteine. However, 90% of the Cu is bound to Cp that is made in the liver. Since Cp is an acute phase reactant protein, Cp is not a reliable indicator of Cu status. Receptors for Cp are found in extrahepatic cells.

BLACK MAGIC SUMMARY

The absorption of Fe, Zn and Cu is very inter-related. Many important proteins for transport and storage have been found and characterized. Interactions are very real in the lumen of the GI tract and may be very important for entry into the enterocyte and ultimate crossing in the plasma. The black box now has holes allowing light to shine on the mechanisms of absorption.

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