

# Trace mineral supplementation for the intestinal health of young monogastric anim...

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## Introduction

Monogastric animal production and profitability have relied on genetic improvements, meeting nutritional requirements, and animal health in order to maximize growth performance and ensure improved efficiency. For both pig and broiler production, producers have been working for decades to increase efficiency and reduce the cost of production. Siegel ( [1](#) ) reported that broiler feed conversion ratio from 1985 to 2010 has improved by almost 35% from 2.3 to 1.5, respectively. Similarly, data ( [2](#) ) from the swine industry support an improvement in feed conversion from 3.6 to 2.7 between 1986 and 2016. However, it is believed that there are still improvements to be made based on genetic potential and nutritional interventions associated with intestinal microflora of monogastric animals. The diversity of microbiome is important in digestion of animal feeds in the gastrointestinal tract. This was demonstrated by Frese et al. ( [3](#) ) and Bian et al. ( [4](#) ), who characterized the fecal microbiome of nursing or weanling pigs and determined that gut microbial populations are clearly established based on dietary content consumed. De Rodas et al. ( [5](#) ) recently reported the bacterial microbiome throughout the gastrointestinal tract from birth to market weight to have increased Clostridia and a decreased Gammas proteobacteria with age. Hence, reminding us that the microbiome is not just influenced by feedstuffs but age.

## Industry Management Issues

The swine industry is continuing to evaluate herd efficiency and health as related to standard operating procedures. The weaning period on a swine

farm is probably the most stressful phase for the young pig which often results in disruptive intestinal barrier integrity due to enterotoxigenic infectious such as *Escherichia coli*, and ultimately a reduction in growth performance for the first few days post-weaning often referred to a post-weaning lag. Moeser et al. ( [6](#) ) reported that weaned pigs at an older age (22–24 d of age) were less likely to have an exacerbated immune response from an *E. coli* challenge when compared to pigs weaned at a younger age (16–18 d of age). Therefore, early-weaned pigs that are stressed during the weaning process may have an altered intestinal microflora and permeability. Additionally, Moeser ( [7](#) ) has reported that the sex of the animal, as well as, weaning age affects gut health; therefore, possibly influencing the permeability of the intestinal lining. He notes that it will “ limit or tightly regulate the exposure of environmental antigens (e. g., feed antigens), toxins, and microorganisms to the gut mucosal immune system” ( [7](#) ). At 24 h post-weaning, De Rodas et al. ( [5](#) ) found that relative abundance of Lactobacillaceae was reduced. As important, the introduction of feed from 21 to 33 days of age had more effect on the microbiome than age, changes in type of feed or change in location for the entire study.

Therefore, in order for the swine industry to overcome the weaning stress issues, much research has evaluated the impact of weaning age and weaning weights on growth performance of nursery pigs. Feed additive antibiotics have historically been used to control pathogenic infections in swine production. However, culturally, antibiotic usage for general control of pathogens have been more closely regulated in order to curtail the antibiotic resistance in the human populations ( [8](#) ). Therefore, finding nutraceutical

feedstuffs that can alter gut health and also improve animal performance is essential. Since the challenge of today's modern hog operations who maintain an antibiotic free system is the prevention of gastrointestinal disease especially when the production is located in high swine concentration environment.

The intestinal microbiota of young piglets can adapt quite quickly to dietary changes (liquid diet to a solid diet) or other environmental stresses. During this critical time, intestinal pathogens often thrive resulting in observations of diarrhea in newly weaned pigs. So, any nutritional interventions through trace mineral supplementation to alleviate post-weaning diarrhea of newly weaned pigs will ultimately improve gut health and overall animal well-being.

Unlike pig production, broiler production does not have a post-weaning lag period associated with weaning the piglet from the sow, but post-hatch chicks often experience a delay in access to feed and water due to the time between hatching and delivery to the farm. The yolk sac functions as an internal nutritional reserve, but often newly hatched chicks will have decreased growth performance, gastrointestinal development, and immunosuppression due to water and feed deprivation ( [9](#) - [11](#) ). Zinc is an essential trace mineral for poultry and required for normal growth, reproduction and development of the enzymatic systems ( [12](#) ). The supplementation of dietary Zn has subsequently been introduced into broiler chickens and has been shown to alleviate the loss of intestinal mucosal barrier function ( [13](#) ). Hu et al. ( [14](#) ) concluded that Zn supplemented at 60

ppm improved growth, intestinal micro-flora, gut morphology, and barrier function in broilers.

## **Copper and Zinc**

The biological necessity of trace minerals for growing animals was determined many years ago ( [15](#) ). However, researchers continue to determine the mechanism and impact of their interactions on performance as well as intestinal microbial population. Emphasis in this area of research has increased in recent years due to the increased concern of antibiotic use in livestock production and the need to find alternatives to antibiotics.

The Cu requirement for young nursery pigs weighing 5–20 kg has been established as 5 to 6 mg/kg/d ( [16](#) ). Copper has been reported to provide antimicrobial effects when fed above the requirement in pharmacological concentrations (100–250 ppm). Currently, there is no agreement relative to the mechanism involved, but Shurson et al. ( [17](#) ) reported an acceleration in intestinal cell turnover while Zhao et al. ( [18](#) ) found reduced villus height. Interestingly early work showed that copper sulfate stimulates average daily gain in nursery pigs ( [19](#) – [21](#) ) and today many forms of Cu are effective in stimulating gain Spears laboratory ( [22](#) ) reported that the villus height was greater in the duodenum and reduced in jejunum in pigs fed 225 mg Cu as sulfate compared to control pigs (6.7 ppm Cu). Additionally, duodenal malondialdehyde concentrations followed this same pattern. However, hepatic cytochrome c oxidase assembly protein 17 mRNA was less and expression of antioxidant I mRNA greater in the sulfate supplemented pigs compared to the controls. Pigs fed 225 Cu as sulfate or tribasic Cu chloride

(TBCC) had greater Cu concentrations in the mucosa of the small intestine than pigs fed 5 ppm Cu., and sulfate resulted in higher concentrations in the mucosal duodenum than pigs fed TBCC. The mRNA of duodenal antioxidant 1 was downregulated in sulfate pigs compared to the control pigs while hepatic Cu transporting  $\beta$ -polypeptide ATPase was upregulated in pigs fed 225 ppm Cu vs. controls ( [23](#) ).

These new laboratory techniques may yield information relative to the mechanism(s) involved in performance enhancement via trace mineral supplementation. However, there is not a consistent response to pharmacological Cu suggesting that various mechanisms and interactions on gut health may be involved.

Pharmacological Cu is frequently fed during the early part of the grow/finish phase at 150–200 ppm. It is interesting to note that in the first dietary phase of grow/finish (44 days post weaning; 63 days of age) the dominant operational taxonomic unit classified as *Campylobacter* were negatively correlated with body weight in the De Rodas et al. ( [5](#) ) study while being fed 150 ppm Cu that followed 200 ppm Cu in all nursery diets. *Campylobacter* is a commensal bacterium that is present in pigs at most ages.

Zinc is an essential nutrient for normal development, growth, DNA synthesis and many cellular functions, and the zinc requirement for young nursery pigs weighing 5–20 kg has been established as 80–100 mg/kg/d ( [16](#) ). However, many researchers further evaluating the 3, 000 ppm Zn supplementation work by Poulsen ( [24](#) ) observed that higher concentrations of Zn as ZnO may improve young pig growth performance ( [25](#) ) as well as reduce

scouring ( [26](#), [27](#) ). Carlson et al. ( [28](#) ) and Case and Carlson ( [29](#) ) determined that the feeding of pharmacological concentrations of Zn in the form of ZnO at a rate of 3, 000 mg/kg/d improves growth performance of newly weaned nursery pigs. The observed improvement in growth performance has mainly been attributed to the decrease in the presence of *Escherichia coli* bacteria count ( [30](#) ). Mechanistically, Carlson et al. ( [28](#) ) observed alternations in the duodenum such as deeper crypts and greater total thickness, as well as increased intestinal metallothionein (MT) concentrations in nursery pigs fed diets supplemented with 3, 000 ppm Zn as ZnO. Thus, her work indicates that high concentrations of Zn have an impact on intestinal health. Based on knowledge from human medical research, animal research looked closer at the intestinal pre-infection microorganisms and a decrease in diversity of the gut microbiome after weaning ( [31](#) ). High concentrations of dietary ZnO have been shown to be beneficial for maintaining the stability of the intestinal microflora, to support a large diversity of coliforms in weaned pigs ( [32](#) ), and to reduce the susceptibility of the pigs to *Escherichia coli* infections ( [33](#) ). Li et al. ( [34](#) ) confirmed these findings when feeding 3, 000 ppm Zn as ZnO to 21 d-old weaned pigs resulting in increased mucosal thickness and villous width of the small intestine.

Vahjen et al. ( [35](#) ) determined that 40–42 d old pigs showed no changes in the order level of ileal microbiome when fed 3, 000 ppm Zn as ZnO, but genus level changes were observed. For example, *Streptococcus* increased while *Sarcina* decreased. In addition, Li et al. ( [34](#) ) reported no effect of pharmacological concentrations of Zn as ZnO supplementation for nursery

pigs on the number of *Enterobacteriaceae*, *Clostridia*, and *Lactobacilli* in ileal digesta and feces. In contrast, Broom et al. ( [36](#) ) and Jensen-Waern et al. ( [37](#) ) found that pharmacological concentrations of Zn as ZnO reduce fecal counts of *Lactobacilli* and *enterococci* during the post-weaning period of pigs, but only temporarily. In agreement, Hojberg et al. ( [38](#) ) reported that feeding weaned piglets 2, 500 ppm of Zn as ZnO reduced the MRS counts (lactic acid bacteria) and Rogosa counts ( *lactobacilli* ) for all segments of the gastrointestinal tract. Impact of ZnO fed at pharmacological concentrations on the microbiome cannot be determined in the De Rodas et al. ( [5](#) ) research since 3, 500 was fed the first 8 days after weaning and 2, 000 was fed from days 8 to 22 post weaning to all pigs.

Studies have shown that *Lactobacilli* are considered to have beneficial effects on human and animal health ( [39](#), [40](#) ) due to its antimicrobial activity against microbial pathogens ( [41](#) ). *Lactobacilli* are among the earliest bacteria to colonize the gut ( [41](#) ). The populations of *Lactobacillus* are thought to be found in high populations in weaned pigs. However, De Rodas et al. ( [5](#) ) reported that *Lactobacillaceae* were reduced 24 h after weaning. Studies *in vitro* and in animals have shown that *lactobacilli* may prevent *Escherichia coli* from colonizing in the jejunum and produce substances directed against the enterotoxins resulting in an inhibition of *Escherichia coli* -induced enterotoxin reactions ( [42](#) - [44](#) ). In agreement, Conway ( [45](#) ) and Chan et al. ( [46](#) ) studying the concept of competitive exclusion of pathogenic *Escherichia coli* by lactobacilli in the intestine and urinary tract *in vitro*, respectively, found that the colonization of the *lactobacilli* sterically hindered the adhesion of *Escherichia coli* to the surface.

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Importantly, Sawai ( [47](#) ) reported ZnO inhibits *Staphylococcus aureus* and *Escherichia coli* growth in the intestine. Thus, providing another mode of action of ZnO fed in pharmacological concentrations.

Roselli et al. ( [48](#) ) using cell culture techniques reported that ZnO may protect intestinal cell from *Escherichia coli* infections by inhibiting the adhesion and internalization of bacteria, preventing the disruption of barrier integrity, and modulating cytokine gene expression, but not by a direct antibacterial effect. Feed grade sources of ZnO vary in color, texture, content, and processing method. Also, ZnO sources tested by chick assays ranged in bioavailability from 37 to 93% based on weight gain and tibia Zn ( [49](#) ). Mavromichalis et al. ( [50](#) ) observed that nursery pigs fed ZnO sources with either high (95%) or low (35%) bioavailability did not affect the growth performance and gut morphology during the entire 21-d assay.

Olukosi et al. ( [51](#) ) reported in broilers that form and amount of Cu and Zn affected performance, percent of breast meat, and concentration of hepatic Cu. As seen by Carlson et al. ( [28](#) ) in pigs, villus height and the villus height to crypt depth ratio were higher in the duodenum when broilers were fed a ZnO source. This data suggests less intestinal permeability when utilizing *ex-vivo* Using chambers; Hu et al. ( [14](#) ) observed reduced colonic permeability to mannitol and inulin.

In the pre-ruminant calf, Jenkins and Hidiroglou ( [52](#) ) showed that 700 and 1,000 ppm Zn reduced weight gain, feed intake and efficiency compared to intakes from 40 (NRC recommendations) to 500 ppm. Dosing calves with 40 g Zn/d resulted in neonatal calves recovering from diarrhea 1 day earlier

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than controls ( [53](#) ). Perhaps these findings are a result of the observations of Rodriguez et al. ( [54](#) ) in guinea pigs and shigellosis infected children ( [55](#) ) that the increased intestinal paracellular permeability observed during fasting and malnutrition is prevented by pharmacological Zn. Additionally, Zn has been reported to promote epithelialization and anti-infective in wound healing ( [56](#) ).

The phytate component in feed ingredients is reported to affect Zn solubility and gut pH ( [57](#) ). Additionally, when pharmacological Zn and Cu are fed, the Zn: phytate and Cu: phytate ratios are altered. Soluble complexes are present at intestinal pH values when the ratio exceeds 10: 1, but insoluble complexes are formed when more than one divalent cation per phytate is formed. Hence, the observed effect of pharmacological Zn and or Cu on phytase activity ( [58](#) ) may explain the observed variation in performance outcomes when pharmacological Zn or Cu is fed to pigs. In broilers, Morgan et al. ( [59](#) ) reported that phytase activity impacted Zn concentration in the gizzard and ileum but not the duodenum indicating the importance of not making assumptions on Zn metabolism between species.

Hill's laboratory ( [60](#) - [62](#) ) has explored many of the Cu and Zn enzyme's activity and associated gene expression, but few swine and poultry researchers have determined Zn and Cu biological signals ( [63](#) ) such as transporters even though it has been shown in humans that dietary Zn intake influences Zn transporters in the plasma membrane of the intestine ( [64](#) ).

## Other Impacts on Intestinal Health

The physical process of weaning pigs from the sow regardless of piglet age has been characterized as the decrease in intestinal barrier function of the gut in the newly weaned pig resulting in decrease pig performance caused by increased intestinal permeability creating alternations in intestinal microbial populations as well as inflammation ( [65](#) ). There are several layers of protection in the intestinal lining against pathogens and toxins. The protection is very important during weaning in a baby pig's life due to the physiological stress of separation from the sow and converting to a dry grain based diet. During weaning, villus height decreases and the intestinal lining becomes more susceptible to pathogens causing reduction in growth performance through lower nutrient absorption. A practical solution for the swine industry for years has been using feed-grade antibiotics; however, more recently other dietary nutritional interventions have been researched such as feeding higher concentrations of trace minerals zinc and/or copper.

As noted earlier, Zn has been known to be essential in many biological functions of mammals, such as anti-inflammation, anti-diarrhea, and maintaining epithelial barrier integrity ( [48](#), [66](#) ). Feeding pigs' dietary concentrations of Zn as ZnO greater than the requirement has shown to impact intestinal morphology of growing pigs ( [28](#) ). Subsequent research supports similar findings with feeding Zn to growing pigs improves intestinal microflora and barrier function ( [67](#) ). Intestinal counts of *Clostridium* and *Escherichia coli* in the intestinal segment of the jejunum decreased linearly when nursery pigs were fed dietary concentrations of zinc ( [67](#) ).

Heat, crowding ( [68](#) ), and weaning are stressors ( [69](#) ) known to negatively impact intestinal health, feed consumption and weight gain. Sanz Fernandez et al. ( [70](#) ) reported that pigs exposed to 36°C with ~50% humidity demonstrated increased ileal and colonic permeability that was decreased by feeding a diet containing 220 ppm Zn. It is not clear if the effect on the gut health is totally due to reduced feed consumption, change in diet form, or decreased energy because husbandry practices often confound the research findings ( [71](#), [72](#) ).

## **Iron and Manganese**

The Fe requirement for young nursery pigs weighing 5–20 kg has been established as 100 mg/kg/d ( [16](#) ). Iron is an essential nutrient needed for hemoglobin in red blood cells where most of the body's Fe is found. However, newborn pigs are unique because of their rapid growth and low concentration of Fe in milk and hence need supplementation of 100–200 mg of injectable Fe in the first 3 days of life ( [19](#) ). Before gut closure, these newborn pigs can very efficiently absorb Fe from the intestinal mucosa ( [73](#) ). Oral Fe within the first few hours of life can be absorbed, but must be administered before gut closure to large molecules. Interesting, research with humans suggests that there is a lack of Fe homeostasis in young infants ( [74](#) ). In rats before weaning, pups cannot regulate Fe homeostasis regardless of Fe status, but 10 days later Fe transporters (DMT1 and ferroportin) in small intestine were affected by Fe status. There is no data to suggest if this occurs in pigs and poultry.

Lactoferrin is absorbed across the intestinal cell via the lactoferrin receptor and is found in colostrum, milk, saliva, tears, and nasal secretion as part of the immune system. Its roles include antimicrobial, immunomodulatory, and Fe binding. The number of eosinophils is higher in the intestine of healthy pigs. The migration of eosinophils, which increase during inflammation, is inhibited by lactoferrin. The receptors of lactoferrin in the pig are found on the duodenal brush border of villi, crypt, and within the lamina propria ( [75](#) ) Hence, this important Fe binding protein not only is important in Fe absorption in the duodenum but in controlling bacteria in the gut. The human small intestinal lactoferrin receptor has been cloned in the pig ( [76](#) ).

Iron toxicity has been shown to occur at 600 mg/kg of Fe in 3-10 d old pigs. In addition, growing-finishing pigs only require 40-50 mg/kg of Fe, but typically growing pig diets will contain four to five times more Fe than required. The excessive concentrations of Fe in commercial swine diets, that may be unavailable to the animal, is due to differing Fe bioavailability of Fe in dietary ingredients such as blood meal, dicalcium phosphate, and limestone ( [16](#) , [77](#) ).

There is an interdependency of the transport mechanisms and regulation of Mn and Fe, two transition elements. Both utilize transporters especially divalent metal transporter-1 (DMT-1). Mammals will have abnormal accumulation of Mn when Fe is low in the diet/body and if Mn is excessive or low, Fe homeostasis is altered. For example, Hansen et al. ( [78](#) ) reported that pigs fed high Fe diets had lower gene expression of Fe encoded proteins in the liver (Hepcidin) and duodenum (DMT1) as well as Mn was lower in liver

and greater in duodenum. These results indicate that dietary Fe supplementation may impair absorption of Mn, but not Cu and Zn.

The acidic environment of the stomach and effect of diet usually result in the Fe that reaches the stomach to be in the ferrous form, but as the pH increases and ferric Fe solubility decreases. Most Fe is absorbed in the duodenum, but the remainder goes to the colon where it is utilized by bacteria. There is the potential for limited Fe absorption from the colon. Rats treated with antibiotics had decreased absorption of Fe ( [79](#) ).

Iron is required for most bacteria to flourish since it serves as a co-factor in re-dox reactions, metabolic pathways and of course the electron transport chain reactions. A few bacteria such as *Borrelia burgdorferi* that causes Lyme disease use Mn instead in proteins requiring Fe. Most bacteria have increased viability in the presence of Fe, and an excess is believed to exacerbate most gut infections.

The research data associated with iron supplementation and the impact on gut health is inconclusive at best. Iron is in abundance and often not considered deficient or limiting in today's animal production. There is research that supports Fe supplementation and increases the presence of beneficial microbiota that may improve the overall gut health of the animal ( [80](#), [81](#) ). However, in a review, Lönnderdal ( [82](#) ) reminds us that excessive Fe has a negative effect on growth and microbial health of the gut if animals or humans had adequate Fe before supplementation. There was an increase in the proportion of anaerobes except when Fe supplementation was high. It is thought to occur because high Fe results in an increase in free reactive Fe

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that induces free radical damage in the gastrointestinal track by release of oxygen by Haber-Weiss reaction. This increase in oxidative stress will decrease the strict anaerobes.

The interaction of Fe and Mn may be the most important information that is known about Mn on gut health. In humans and experimental animals, it has been shown that Mn is not well-absorbed, and it appears from experimental animal studies that fiber, phytic acid, oxalic acid, Ca and P reduce its availability for absorption ( [83](#) ). Perhaps more importantly, Mn and Fe compete for binding sites that effects absorption and ultimately body stores ( [84](#) ).

It is believed that Mn absorption in poultry is less than pigs, but there is no definitive research with today's genetics in either species. Using polarographic analysis, solubility in buffers, and deionized water, Li et al. ( [85](#) ) reported that bioavailability of five different organic Mn sources was closely related to chelation strength. While organic Mn has been promoted to be of value in preventing leg abnormalities, this incidence of swelling of the tibio-tarsal joint abnormality is not prevented by Mn dietary supplementation. This broiler problem is more severe if poly-unsaturated fatty acids are added to the diet for growth promotion.

When inorganic and chelated minerals (Cu, Zn, Fe, Mn) fed at reduced dietary concentrations were compared to control fed pigs from weaning to finishing, reduced mineral concentrations regardless of source did not affect performance but resulted in reduced fecal excretion ( [86](#) ). Besides the role of Mn in metabolic functions of enzymes, supplementation of Mn has been

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reported to result in greater lean color scores and more vivid red color in pork chops when provided as a sulfate but not an organic form ( [87](#) ). This might indicate that absorption differed perhaps due to valence changes.

## **Conclusion**

Health of the gut is reflected in the performance of the animal from hatch/birth to death. Additionally, changes in the gut's morphology may not provide a means of evaluation of gut health. However, it is currently being used with the *ex vivo* studies with Using Chambers that determine the capacity of the intestine to transport nutrients. Hence, the techniques and technologies currently being used in research today will give additional information lacking in today's literature. Clearly, the published National Research Council's nutrient requirements for specific species should only be used a guidelines. However, researchers have determined that any disruption of the gastrointestinal tract will impact animal performance, and supporting the antioxidant system by the usage of trace mineral supplementation will ensure intestinal microbiota health and repair. In conclusion, much of the results of positive and negative attributes of trace mineral supplementation on gut health are influenced by genetics, production goals, and the environment.

## **Author Contributions**

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.



## Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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