New Frontiers: Premix Interactions, Epigenetic Effects, and Antimicrobial Resistance

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There is an old adage among animal nutritionists that there are three different diets: “the diet that was formulated, the diet that was mixed, and the diet that was actually eaten.” This is usually used to point out how easy it is for the perfectly formulated ration to end up with disappointing results. It might not be surprising that there are plenty more places for the “perfect” ration on paper to result in unexpected outcomes. In this paper, we take a brief look into three relatively new areas of research that show that the path from ration formulation to ultimate performance in populations of animals is highly complex and fascinating. This serves as an example of just how much there still is to learn and improve in the efficiency and long-term sustainability of our critical animal-based food production systems.

1. Premix and feed antagonism

Increasingly, the agonistic and antagonistic effects of feed components have come under scrutiny, with choice of components gaining increasing importance in diet formulation. The possibility for negative interactions occurring between individual components within premixes and feeds is high and often overlooked, as are the underlying effects at a cellular level following digestion and absorption of the mineral source.

Recent studies have focused on assessing these potential antagonisms. The differential effects noted indicate that not all chelates are created equal. Moreover, they all differ in terms of their stabilities, releasing mineral in a pH-dependent fashion based on the pH in the local microenvironment. This instability results in some chelates having a negative impact on premix and feed components.

1.1 Effect of minerals on enzyme activity

Very little information is available comparing the potential antagonisms that can occur between different mineral sources and enzymes within premixes, as well as the repercussions that this might have in terms of losing enzyme efficacy.

Santos *et al*. (2014) focused on assessing the potential in-vitro interaction between inorganic and organic chelated sources of Fe, Zn and Cu with three commercially available phytase preparations as a model. The study also investigated if the degree of enzyme inhibition was dependent on the type of organic trace mineral (OTM) used as a mineral source.

The authors demonstrated that a highly significant relationship between phytase inhibition and trace mineral type, as well as mineral source and concentration, existed.

Proteinates were consistently and significantly less inhibitory than the other mineral sources, and this was shown in the case of the *Escherichia coli* and *Peniophora lycii* phytases for Fe and

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Zn, as well as for Cu with *E. coli* and *Aspergillus niger* phytases. The dose-response curve illustrating the impact of Fe source on *P. lycii* phytase activity is shown in Figure 1.

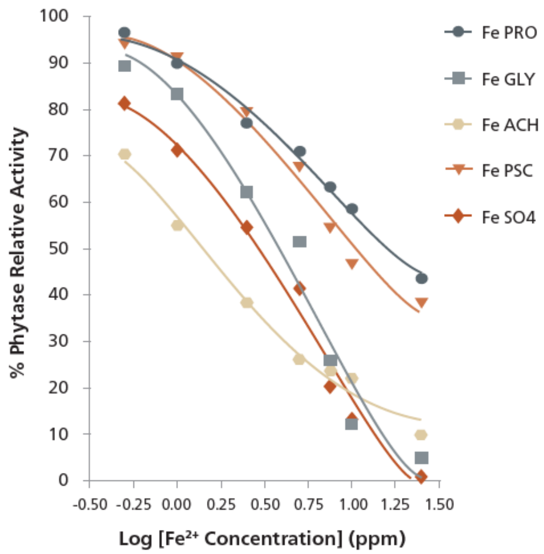

Figure 1: Sigmoidal dose-response curves representing the effect of Fe sources on *P. lycii* phytase activity (Santos *et al*., 2014).

Overall, different OTM sources displayed differential effects in their inhibition of exogenous phytase activity. The consequences that this mineral-induced inhibition of enzyme activity has for premix and feed formulation are tremendous and go some way towards explaining the variation noted in supplementation response. There is also preliminary information that these effects exist for endogenous enzymes as well, which have the potential to affect the way the body digests the ration that ends up being consumed.

1.2 Effect of minerals on vitamin stability

Vitamin oxidation and antioxidant function are primarily caused by autooxidation of fats (a phenomenon that can be self-propagating) or by trace minerals through Fenton-type oxidizing reactions. In trace mineral premixes, oxidation-reduction reactions are the predominant cause of vitamin instability.

The type of trace mineral will influence its reactivity with copper, iron and zinc being the most reactive and having the greatest potential for vitamin destruction. A recent study (Concarr *et al.*, 2021) illustrates these effects nicely. The study, which examined vitamin E stability following short-term inclusion in mineral premixes containing inorganic sulfates or different forms of organic minerals, demonstrated that mineral form significantly influenced the stability of α-tocopherol (Figure 2). Vitamin E stability in the premixes containing proteinated chelates was not significantly different when compared to the vitamin control. The two premixes that had the highest α-tocopherol acetate loss were those containing the amino acid complex (25.7% decrease) and the glycinate (31.9% decrease). Both premixes were noted to have higher losses than were found within the vitamin control and the proteinate source. These data demonstrate the importance of carefully choosing premix components. An additional study by Concarr *et al.* (2021b) further examined the destabilizing impacts of mineral form on vitamins in premix. The authors found that both retinol acetate and cholecalciferol stabilities significantly increased ($P \leq 0.05$) within vitamin-trace mineral premixes through the inclusion of chelated mineral. The data indicated that enhanced levels of trace mineral increased retinol acetate and cholecalciferol degradation in line with the duration of time in storage, but that losses could be minimized by switching from inorganic to organic forms. This is illustrated in Figure 3, where the impact of mineral form and level on vitamin D3 stability is apparent.

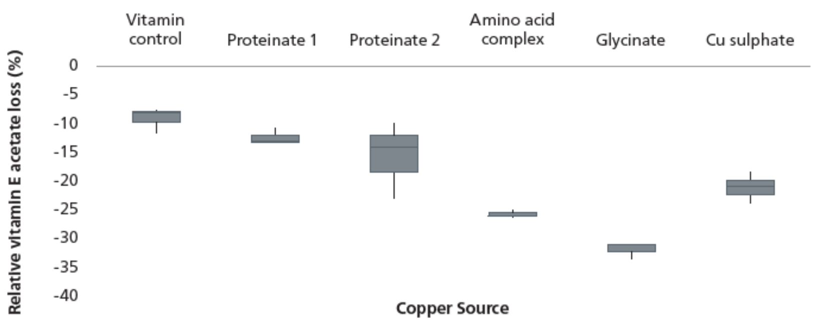

Figure 2: Vitamin E instability in premixes is influenced by mineral form (Concarr *et al.,* 2021).

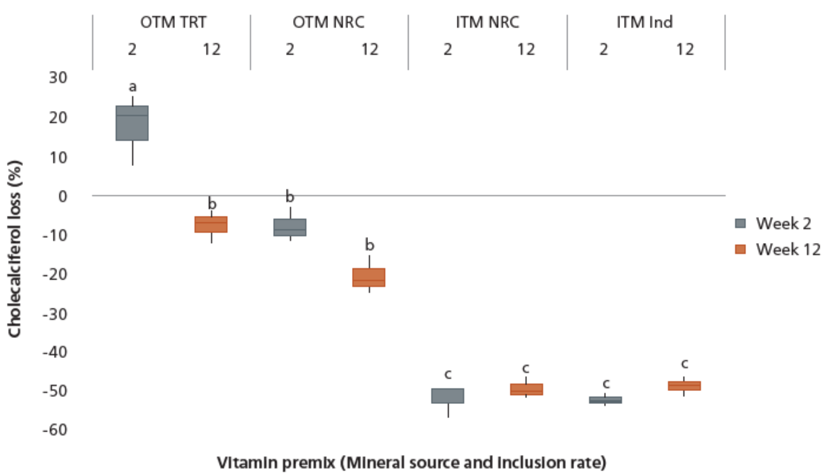

Figure 3: Impact of mineral form, level and storage time on vitamin D3 stability in premix (Concarr *et al.,* 2021b).

1.3 Impact of mineral form on antioxidant efficacy

Additional research by the same author (Concarr *et al.,* 2021) assessed the effect of mineral form in reducing the efficacy of recognized feed antioxidants such as butylated hydroxytoluene (BHT) . This study compared inorganic copper sulfate to different organic mineral sources of copper (glycinates, amino acid chelates and proteinates). The results further indicate that the efficacy of commonly used premix components, such as antioxidants, can be compromised using inorganic trace elements (Figure 4). The data further indicate that, in some cases, organic trace elements also had a significant destabilizing impact on antioxidant function. Essentially, weakly bonded minerals may result in the liberation of free mineral ions, causing reactive oxygen species generation, which leads to greater oxidation and a reduction in the efficacy of feed antioxidants such as BHT.

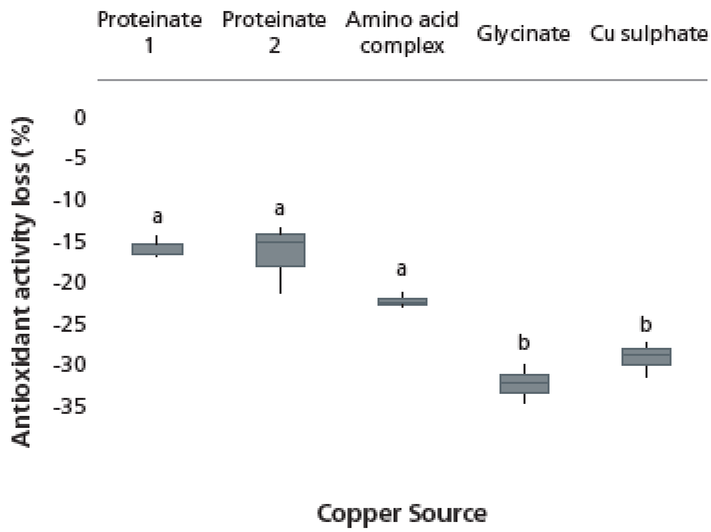

Figure 4: Impact of mineral form on antioxidant (BHT) efficacy (Concarr *et al*., 2021).

2. Epigenetic effects

Epigenetics is a field that is still in its infancy. Generally, epigenetic effects refer to heritable changes in gene expression caused by mechanisms other than the changes in the underlying DNA sequence. From a nutritional standpoint, this practically means that what you feed the sire and, especially the dam, can affect the eventual phenotype of that offspring, regardless of genetics: “You are what your mother ate.”

While most nutritionists recognize that maternal nutrition will affect the eventual phenotype of the offspring, there is not a large amount of data demonstrating how nutritional interventions during pregnancy can modify the resulting phenotype of the offspring. Here, we look at one beef and one dairy study showing practical examples on how trace mineral nutrition of the cow can affect the resulting offspring.

A study on beef cattle out of the University of Florida (Price *et al*., 2016) and a dairy study at Penn State University (Pino *et al*,. 2018; Gelsinger *et al*., 2016; Pino and Heinrichs, 2016) showed remarkably consistent responses to a trace mineral program that focused on the total replacement (TRT) of inorganic trace minerals with proteinates (Bioplex®) and selenium yeast (Sel-Plex®).

From the two studies, the primary performance indicators (milk production for dairy cows, weaning weight for beef operations) are driven by direct supplementation with the TRT organic mineral program. Immunity of the offspring was determined to be improved both by maternal supplementation of the trace mineral program, or supplementation of the calf independently. Fertility indicators were also improved in both studies.

However, age of puberty and thus average days confirmed pregnant and age of heifers at first calving, was not affected by the diet on which heifer calves were raised. Instead, the primary determinant of these parameters in both studies was the supplementation program of the dam. Total replacement of inorganic minerals resulted in puberty as much as 41 days earlier in the beef herd and age at first calving of 26 days earlier in the dairy herd (Figure 5).

It is exciting to consider that many such effects of maternal nutrition remain unstudied and, therefore, undiscovered.

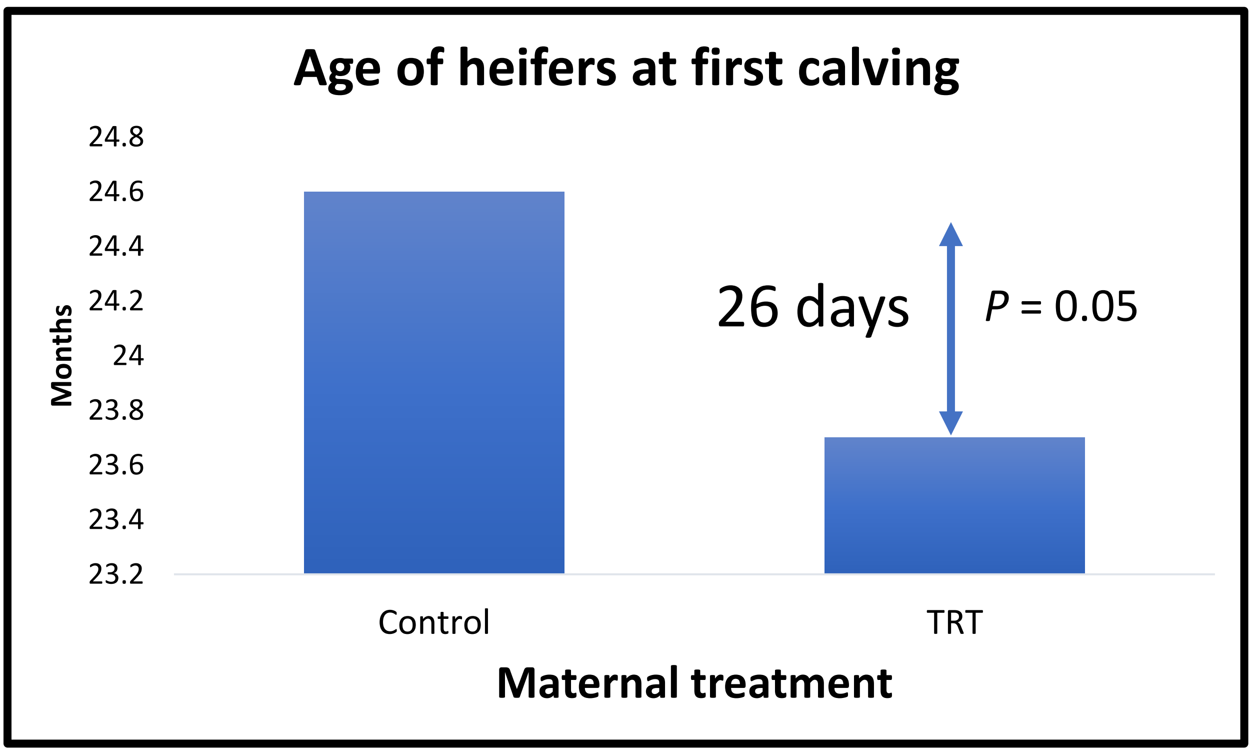

Figure 5: Maternal effect of organic trace mineral supplementation on age at first calving in heifer offspring.

3. Antimicrobial resistance

Antibiotic resistance has the potential to become one of the greatest problems of our generation, given the ever-increasing rise in bacterial strains that are less and less sensitive to existing treatments. While abuse of antibiotics in humans is probably the major contributor, policymakers have turned the spotlight on agricultural use as a way to control the problem. Globally, it is recognized that there is no so-called “silver bullet” to replace antibiotic use in animal production and producers will almost certainly have to improve hygiene and husbandry to address the issue.

Products that will assist the move to antibiotic-free production status include many that are designed to regulate and support the gut environment and its microflora, such as:

- Coccidial vaccines
- Probiotics
- Competitive exclusion products
- Feed enzymes
- Functional nutrients, such as nucleotides
- Organic acids and feed hygiene products
- Organic minerals
- Plant-based products, such as herbs, spices and essential oils
- Yeast cell wall derivatives, such as mannan-oligosaccharides (MOS) and mannose-rich fractions (MRF)

Of the functional ingredients currently in use for microbial control, MOS are widely used in animal nutrition and have been shown to improve animal performance in a manner similar to antibiotic-like growth promoters. While the early use of yeast mannan products was linked with control of pathogens such as *Salmonella* and *E. coli*, further refinements of yeast MOS have led to the isolation of a mannose-rich fraction (MRF) with enhanced benefits for intestinal health.

Recent studies on MRF have focused on the impact that these more refined carbohydrate fractions have on the overall bacterial community of the intestinal ecosystem. Such work has shown that MRF supplementation can significantly enhance the diversity of the intestinal microflora (the so-called microbiome), and in doing so, decrease the prevalence of microbial pathogens, such as Salmonella and *Campylobacter*, which are of interest to human health.

In terms of developing strategies to reduce or limit the use of antibiotics, perhaps an elegant solution is to find ways to make the therapeutics more effective. MRF supplementation of the diets of broilers has been associated with a decrease in selected antibiotic resistance gene copy numbers. This is potentially linked to the ability of MRF to reduce plasmid transfer between microbes (Figure 6), and in doing so, prevent the spread of antimicrobial resistance (Smith *et al.*, 2020).

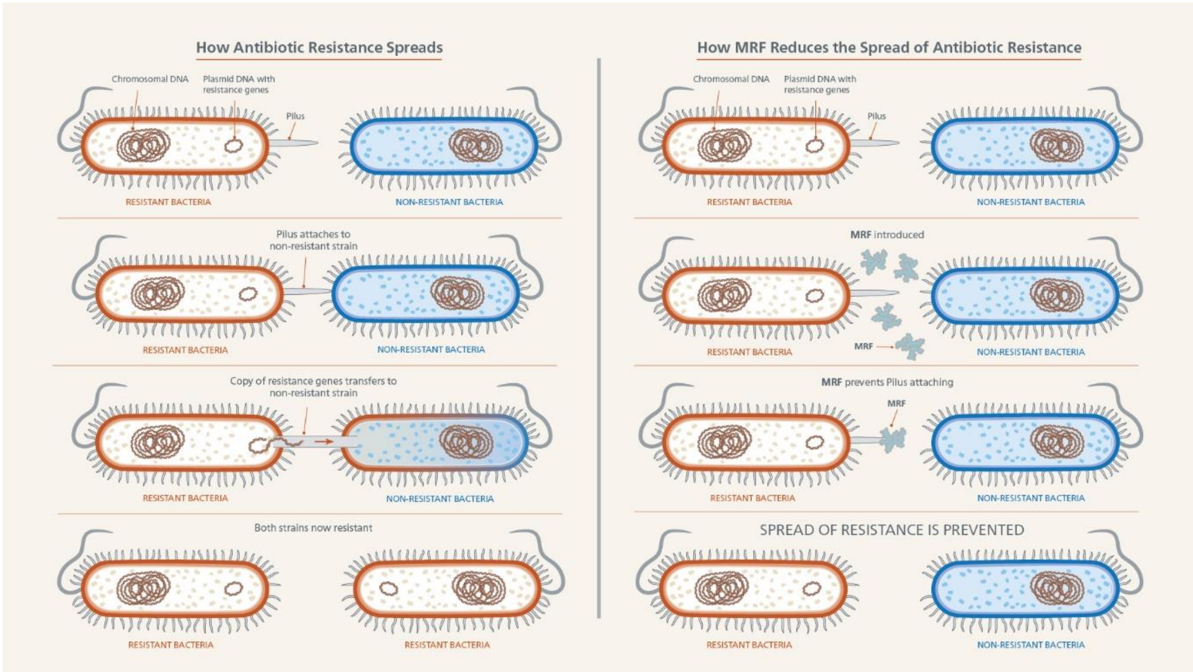

Figure 6: MRF reduces spread of antimicrobial resistance.

Additional research has shown that MRF can influence bacterial metabolism and, in doing so, influence the sensitivity of resistant bacteria to antibiotics. This new research demonstrates that when resistant *E. coli* is grown in the presence of MRF, its growth and metabolism are altered, resulting in antimicrobial-resistant strains becoming increasingly sensitive to antibiotic treatment. By enhancing the sensitivity of bacteria to the effects of antibiotics, we can potentially reduce the minimum inhibitory concentration (MIC) required.

From a production standpoint, it is essential that any moves toward antibiotic-free production systems improve overall feed quality, as animals that are fed quality feeds are less susceptible to enteric problems. Ultimately, this move from least-cost feed formulation and reliance on antibiotics will be toward high-quality feeds containing functional ingredients.

Programs that employ a holistic approach to health management have proven to be extremely effective. By utilizing a combination of strategies, producers can rehabilitate and accelerate the evolution of the intestinal microbiota. The success of these “Seed, Feed and Weed” programs is reliant on first seeding the gut with favorable microflora using a probiotic, feeding the favorable microbes through acids or enzymes and, finally, weeding out pathogens by using MRF products.

Ultimately, concerns among scientists, regulators and consumers about antibiotic resistance have driven the EU ban on antimicrobial growth promoters (AGP) and been a catalyst for change in the U.S. This has heralded a global move to reduce antibiotic usage, and further ongoing changes in animal production systems are likely to be substantial. Ultimately, what is required are innovative replacement products and alternative strategies. The use of functional feed components, such as MOS and MRF, represents one such innovative approach to break the cycle of resistance.

4. References

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