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Richard H Bristol, MS
Director of Nutrition and Technical Services
ILC Resources
3301 106th Circle
Urbandale, Iowa 50322-3740
(515) 243-8106
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www.ilcresources.com

richardb@ilcresources.com

Interactions Among limestone-dical-phytase Treatments of Corn/Soybean Meal Affect Calcium & Phosphorus Solubility

Pieces of the Puzzle:

We've used *Mineral Writes* not only as a venue for the conveying of information and the sharing of ideas, but also as a means to challenge our livestock/poultry feeding industry toward further discoveries of how pieces of this exciting **calcium** puzzle fit together into a more complete picture. Our goal, of course, is to have the articles reviewed in this venue fit pieces into the puzzle and reveal a clearer, fuller picture. Occasionally, we may be left wondering whether this has been accomplished. Parts may fit, but the puzzle remains a cloudy picture yet. Just as this imagery denotes, pieces may fit without revealing a clear enough, complete enough picture. Additional discovery fit to current information may unlock better understanding. Such may be the case in this review.

The roles calcium (Ca) plays alone and in relationship with phosphorus (P) have been examined for many decades and have been reported in numerous research studies. Yet we still do not know nearly enough about how they work together to optimize both health and performance in animals. Undeniably, calcium is necessary for eggshell production in poultry. But the vast majority of calcium requirements are centered on bones and teeth formation. Proper skeletal development is of paramount importance for growing and finishing of meat production animals along with proper maintenance of skeletal integrity in breeding animals. Milk production in dairy cows needs ample Ca & P to meet nutritional demands targeting human food markets. Calcium and phosphorus also play a major role in milk production for progeny rearing in most species. Ninety eight to 99% of the body's calcium is deposited in the bones. In conjunction, some three quarters of the body's demand for phosphorus ends up in the skeletal system along with calcium. As more knowledge is acquired about calcium-phosphorus dynamics, even more questions arise calling for additional research.

***In Vitro* Research:**

A recent study illustrates the need for deeper Ca-P understanding and points out how complex Ca-P dynamics are. The density of confounding factors involved in this laboratory study is certainly indicative of reality happening in the animal. Let's take a look.

In the March 2012 issue of the *Journal of Poultry Science* an article reported findings from an *in vitro* evaluation of dynamic interactions among limestone, dicalcium phosphate, and phytase affecting calcium and phosphorus solubility during digestion of corn and soybean meal (SBM). A team of researchers from the *United Kingdom* and *Virginia Tech* undertook an intricate study to evaluate the influence of limestone, dicalcium phosphate, phytase, and the digestion phases on Ca and P solubility. Laboratory trials were set up with experimental samples arranged in a 2 X 2 X 2 X 2 factorial containing corn or soybean meal plus limestone, dicalcium phosphate, phytase, and all combinations. Further, these samples were exposed to a 2-step *in vitro* digestion assay to simulate both gastric and small intestinal phases of digestion. Much detail is necessarily omitted in our review here in favor of presenting highlighted points. If the reader's interest is piqued for more depth, we refer the reader to the actual report as found in *2012 Poultry Science 91:674-682.*

Defining Solubility:

We need to pause a moment and examine the terms *soluble* and *solubility*. For a substance to be *soluble* it must be "…capable of being dissolved, especially easily dissolved." Further, *solubility* is defined as "…the amount of a substance that can be dissolved in a given amount of solvent." Throughout this discourse, realizing that this phenomenon may take on more than one application will help immeasurably with understanding this research.

To begin with, solubility of a substance may first apply to the breaking down of foodstuffs in the body into a form that can be absorbed, which actually defines digestion. In the case of ingested limestone (calcium carbonate – $CaCO_3$), this supplemental source of calcium is dissolved in the hydrochloric acid (HCl) of the stomach. That dissolution results in breaking down this substance to release ionized Ca^{++} to be available for absorption. The key here is to recognize that the substance (*feed-grade* $CaCO_3$) is dissolved in the acidic gastric -- HCl. Subsequent absorption of freed Ca^{++} must take place further down the GI tract in the small intestine as it remains in its solublized state. Essentially, this pathway also describes the dissolution of dicalcium phosphate during digestion in gastric HCl yielding ionized phosphate (PO_3^-) for subsequent absorption.

Now, the question remains, "What about the solubility of Ca and P regarding absorption in the small intestines?" Their sources have already been dissolved; will these free ions remain solublized? Both concepts use *solubility* in their descriptions, but we need to keep in mind one pertains to the supplemental source (i.e. limestone and/or dical) and the other pertains to solublized Ca or P in their ionic states. If either Ca or P precipitates out of solution by reacting with another reagent, its ability to stay solublized for absorption and subsequent utilization is reduced.

Procedures and Findings of the *UK, Virginia Tech* Study:

"The present series of in vitro studies was designed to evaluate the availability of Ca and P in corn (EXP1) or soybean meal (EXP2) in the presence or absence of phytase, limestone, and dical

at the gastric and SI (*small intestine*) phases of an *in vitro* digestion assay. The experiments evaluated the hypothesis that phytase improves Ca and P solubility in the gastric phase of digestion and subsequently improves the Ca and P solubility in the SI by eliminating phytate precipitation."

The eight treatments for each ingredient were as follows (Table 1):

Table 1	Treatment Samples							
Ingredient	1	2	3	4	5	6	7	8
EXP1	% in Sample							
Corn	100.00	97.45	98.01	99.98	96.60	98.70	97.43	96.88
Limestone	0.00	2.56	0.00	0.00	1.40	0.00	2.56	1.81
Dical-18.5	0.00	0.00	1.99	0.00	2.00	1.29	0.00	1.30
Phytase	0.00	0.00	0.00	0.02	0.00	0.02	0.02	0.02
Calculated Composition:								
% Ca	0.03	1.00	0.47	0.03	1.00	0.31	1.00	1.00
% Total P	0.25	0.24	0.61	0.25	0.61	0.48	0.24	0.48
% Available P	0.08	0.08	0.45	0.21	0.45	0.45	0.21	0.45
% Phytate P	0.17	0.17	0.17	0.17	0.16	0.17	0.17	0.16
EXP2	% in Sample							
Soybean Meal	100.00	98.25	98.80	99.98	97.73	99.49	98.23	98.01
Limestone	0.00	1.75	0.00	0.00	1.05	0.00	1.75	1.46
Dical-18.5	0.00	0.00	1.21	0.00	1.22	0.50	0.00	0.51
Phytase	0.00	0.00	0.00	0.02	0.00	0.02	0.02	0.02
Calculated Composition:								
% Ca	0.34	1.00	0.60	0.34	1.00	0.45	1.00	1.00
% Total P	0.69	0.68	0.90	0.69	0.90	0.78	0.68	0.77
% Available P	0.23	0.23	0.45	0.36	0.45	0.45	0.36	0.45
% Phytate P	0.37	0.36	0.37	0.37	0.36	0.37	0.36	0.36

Important to note is that this was an in vitro digestion study and not treatment diets being fed to experimental animals. What was measured in this trial was the effect the three basic ingredients had on the nutritional factors of Ca, P, and phytate when combined with either corn or soybean meal. [Obviously, in an *in vivo* study, researchers would not have been able to separate dietary components to examine specific modes of action individually.] It was apparent, of course, that Ca and P levels were not equalized across treatments. Phytase treatments were standard at 1000 units/Kg sample. The above treatments were measured in both gastric incubation phase (~pH = 3) and SI phase (~pH = 6). In pullets and chicks these pH ranges follow gizzard & proventriculus levels (pH = 2) and duodenum levels (pH = 6) pretty closely. Thus, experimental conditions mimicked gastrointestinal tract environments. Both nonsignificant interactions and main effects of treatments were not reported for the sake of only covering the presence of significant interactions.

In both experiments Ca and P solubility were influenced by interactions of dical, limestone, phytase, or digestion phases. One needs to keep in mind that Ca, P, and phytate contents among treatments varied dramatically. For example, in EXP1 corn samples Ca:P ratio varied from 5:1 in limestone only treated samples to less than 2:1 in limestone-dical treated samples to even reversed Ca:P ratios in samples without any limestone supplementation. Similar trends existed among soybean meal samples in EXP2. Another point, phytate P in corn is 0.17%; whereas phytate P in soybean meal is 0.37%. Therefore, the Ca:P ratio and Ca, P, and phytate P contents of the treatments are confounded by the presence or absence of the specific ingredients.

Limestone X dical X phytase X digestion phase (either singly or in combinations) influenced Ca solubility in EXP2 with SBM, but no significant interactions affecting Ca solubility were observed accordingly in EXP1 with corn. Predictably, Ca was more soluble in the gastric phase than in the SI phase, but was not influenced by dietary ingredient. At low acidic pH during gastric phase, Ca, phytate, and P are soluble and less likely to precipitate out. However, an increase in pH as in SI phase would promote Ca, phytate, and P precipitation and reduce the solubility of Ca and

P, which the findings revealed. In EXP2 (SBM) without limestone, the total Ca content of the samples was approximately 50% lower than the total Ca content was in the samples with limestone. An extremely low Ca:P ratio may promote calcium phosphate precipitation. Supplementation with dical and phytase alone brought Ca:P ratio closer to 0.7:1, and free phosphate would precipitate with Ca, thereby reducing Ca solubility as well as phosphate P solubility. Phytase alone hydrolyzed phytate, thus increasing Ca solubility and phytate P. "In the presence of dical and phytase, the phosphate from dical and phytate hydrolysis would promote the creation of insoluble calcium phosphate bonds. Therefore, the presence of phytate or phosphate can both precipitate Ca and interfere with Ca solubility."

EXP2 with SBM showed influences of phosphate formation by (a) absence of limestone creating low Ca:P ratio promoted calcium phosphate precipitation, (b) release of substantial P from high phytate concentrations by phytase, and (c) phosphate addition through dical supplementation. These influences on Ca solubility in SBM were not noticed in corn, which may be due to lower phytate P content in corn versus in SBM. Even without added limestone, Ca:P ratio would be higher in corn than in SBM. Also, less phytate P present in corn would substantially reduce the availability of phosphate to precipitate out with Ca to form Ca-phosphate bonds even with dical supplementation. Thus, little or no reduction in Ca solubility is observed in corn.

In corn (EXP1), however, Ca solubility was influenced by interactions of limestone X digestion phase, limestone X dical supplementation, limestone X phytase treatment or dical X phytase treatment. Higher Ca from these interactions essentially promoted phosphate or phytate precipitation of Ca actually reducing Ca solubility in both the gastric and SI phases with more precipitation occurring at the higher pH range in the SI. In limestone supplemented treatments, the absence of dical created a large Ca:P ratio, which promoted Calcium phosphate precipitation and reduced the amount of soluble Ca. Researchers studying broilers in 1995 saw Ca retention was reduced as the dietary Ca:P ratio increased from 1.1 to 2.5:1. Improving Ca solubility by supplementing both limestone and dical was presumably the result of a reduced Ca:P ratio and a balance in soluble Ca and P, thus reducing calcium phosphate precipitation. This study speculated that the presence of Ca from limestone without the inclusion of P from dical created a large Ca:P ratio and (a) reduced efficacy of phytase, or (b) promoted phosphate precipitation, thereby reducing P solubility in the SI phase compared with the gastric phase.

In this trial, "…soluble P in SBM in the gastric phase was significantly higher in the presence of dical, limestone, or phytase compared to diets without limestone, dical, or phytase." However, the solubility of P in the SI phase was significantly reduced due to calcium phosphate or calcium phytate precipitation at the higher pH of the SI phase. Phytase supplementation in SBM slightly improved P solubility in the SI phase, presumably due to an increase in free phosphate being released from phytate or dical, but P solubility was still significantly lower than solubility in the gastric phase.

Reaching Some Conclusions:

The study concluded that solubility of Ca or P is controlled by the amount of total Ca, total P, and phytate available to precipitate free Ca or free P. An imbalance

in the Ca:P ratio, including an excess of phosphate above that of Ca, will cause calcium phosphate precipitation, especially in the SI phase, and could even reduce digestion of other key minerals. Factors that influence the solubility of Ca, P, and phytate are essential in determining nutritional adequacy in dietary formulations. The study's final comment was, "Therefore, the solubility and presumed availability of both Ca and P is dependent upon a multitude of factors, and as a result of the above, it is clear that precise Ca and P nutrition is an extremely difficult target but if achieved, could yield benefits beyond mineral nutrition."

From these reported results, presented in both narrative and table data, this study suggests that influences on solubility of both Ca and P are best achieved when both treatment levels are optimized. But, even this assumption needs to be tempered by interpreting not only dietary sources of Ca and P but also properly achieved Ca:P ratio. When individual Ca or P treatments were supplemented, the resultant solubilities were highest. On the other hand, when either Ca or P was properly supplemented and the other was not, the combined solubility values were compromised simply due to the obvious imbalance of Ca:P ratio. One observation of merit suggests that P supplemented in both experiments (corn & SBM) resulted in the best achievement if coming from inorganic dical sources along with limestone furnishing proper levels and ratios of Ca to P. This would maybe give pause to reconsider dynamics of maintaining both inorganic sources of Ca and P from $CaCO_3$ and dical in diet formulations. Certainly, the soundness of phytase addition to diets unlocking phytate P is undisputed, but maybe formulations to the point of exclusion of one source of P versus the other could be unwise. Above all, it appears that adequacy of supplemental Ca and P levels is just as important as adequacy of relationship of one to the other resulting in proper Ca:P ratio. Otherwise, imbalances in both areas reduce performance as this study of *in vitro* Ca & P solubilities demonstrates.

Applying What We've Learned:

The dynamics of Ca and P nutrition presented in this study are challenging to track. Our minds want to follow less complicated avenues as we seek explanations of nutritional dynamics. For example, usually diet formulations are carried out first considering gross nutrients of protein (i.e. amino acid profiles) and energy and bringing in such basic ingredients as soybean meal and corn to balance these needs. Somewhere following these are mineral considerations. Based on the compositions of Ca and P in ingredients such as corn and soybean meal, the nutritionist is likely to pull in two or three more ingredients to finish balancing the Ca and P requirements. Predictably, they might include dical (expanded to either 18.5% Dicalcium phosphate or 21% Monocalcium phosphate), limestone (**Calcium Carbonate – $CaCO_3$**), and/or phytase enzyme (to unlock phytate P). The study just reviewed certainly points to differing dynamics that these three basic supplemental Ca & P sources exhibit as *different* dietary ingredients are used. As we look at more complex diets today, how will broader based dietary ingredients beyond simply corn and SBM react? We may face more questions than we even know we need to consider. Will ethanol by-products follow corn dynamics presented here? Or will resultant higher nutrient concentrations follow some similar course as SBM? Or, will some different interactions be revealed demanding

another set of considerations? Or could it all be as simple as considering these key mineral nutrient concentrations alone in the ingredients to be used and adjust diet formulations accordingly? What options will need to be considered in future dietary formulation software programs accordingly? Questions will be asked and answers will be sought as needed.

We simply do not know enough about such heretofore simple nutritional factors as *calcium* and *phosphorus.* From ILC Resources' perspective, we know *limestone* is far more complicated and should foremost be understood as **$CaCO_3$** of varying particle size gradations, which definitely affect the acid/base reactionary release rates of Ca^{++} defining inherent bioavailability and absorption. Our responsibility and focus are on testing, defining and understanding our various products to assist the livestock/poultry feeding industry in making informed decisions about products to use for a variety of feeding conditions involving a variety of species. We know we can provide some answers, but we also continue striving to seek greater understanding.

Continuing Role of ILC Resources:

One such addendum to this report comes from a research study at the University of Arkansas (published 3rd Qtr *Mineral Writes* 2006). That study looked at performance in broilers supplemented with a range of particle size gradation $CaCO_3$ products. Both extremes of high and low particles were detrimental. Coarse particulate $CaCO_3$ solublized Ca^{++} too slowly to provide adequate dietary Ca to meet requirements. However, even more detrimental was supplementing the diet with finely ground powdered $CaCO_3$. This material tested high by *in vitro* acid solubility released free Ca^{++} too rapidly presumably causing Ca to rebind the phytate molecule; thus, preventing the release of not only phytate P but Ca as well. Granular particles of supplemental $CaCO_3$ optimized performance. Presumably this allowed dietary phytase to release P and Ca fully from the phytate molecule *(below)* without rebinding, plus furnish more timely release of additional Ca necessary to meet requirements for growth. This exemplifies another piece of the puzzle fitting together.

Genetic improvements in livestock and poultry accelerate both performance and efficiency of production. Accordingly, there has been an evolution of what we feed and how we feed our animals better in order to capture optimum performance and efficiencies. However, as far as these improvements have brought us from our past knowledge and practical applications, we are even more excited to look ahead toward greater understanding and improved applications for the future of our industry. We are unlocking mysteries while at the same time we discover additional challenges. Let's stayed tuned.