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#### Limestone Solubility: What Can You Do About It?

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#### **Background of limestone solubility survey**

Calcium (**Ca**) is essential for bone development mineralisation, as well as for eggshell formation, muscle and neural functions. Depending on the type of diets (veggie vs. non-veggie), species (turkey, chicken, duck), and age of animal (young vs. adult), limestone can contribute up to 95% total Ca in the final diet, with more Ca from limestone for younger animals with veggie diet. Despite the significant contribution of limestone to total diet Ca levels, the bioavailability variation of limestone Ca is rarely considered.

Phosphorus (**P**) is a limited resource and is the third most costly ingredient in poultry diets. Improving P utilization in poultry diets will decrease the amount of inorganic P needed, reduce P excretion and feed costs, as well as increase the sustainability of poultry production. Studies in the past two decades have clearly demonstrated that Ca can be detrimental to P utilization. Some recent studies suggested that phytase, if used correctly can partially or even completely alleviate such detrimental effect. In addition, recent evidences also showed that formulating on bioavailability of Ca, instead of total Ca, could be more favourable in regard to feed cost, animal performance, and sustainability.

The global limestone survey started in 2018, primarily to understand the variation of: 1) Ca concentration from the most significant Ca source and probably the cheapest ingredient limestone; 2) solubility and potentially bioavailability of the Ca from limestone. In parallel, a series animal studies were carried out to determine: 1) correlation of *in vitro* limestone solubility and *in vitro* Ca digestibility; 2) the impact of solubility profile on P digestion and phytase efficacy. It has been four years since the survey started, with over 1500 samples collected globally. It is a good time to review the results from and learnings so far.

#### **Progress and status**

At the time of report, 1772 samples have been received, analyzed and documented, of which, 1036 samples are fine limestone (<1000  $\mu$ m) representing 60% of the total samples, rest are coarse limestones (>1000  $\mu$ m). There are 212 samples (130 fine limestone, 82 coarse limestone) from the US, accounting for 12% of the global samples received.

For the fine limestones, average geometric mean (**GMD**) was 396  $\mu$ m (median = 366  $\mu$ m). About 90% of the fine limestone samples received were smaller than 670  $\mu$ m, and 10% were below 100  $\mu$ m, which can be considered "powdery limestone". The mean and median Ca concentration was 37.98 and 38.36%, respectively. Out of the 90% samples that fell under 670  $\mu$ m, only 32% could be considered "good limestone", as defined by the collecting criteria referred from animal and *in vitro* trials: 1) Ca content (>38%), 2) solubility (<65%) at first timepoint, and 3) solubility (>85%) at 3<sup>rd</sup> timepoint from *in vitro* incubation. Limestones that failed to meet those criteria could potentially be: 1) more detrimental to P digestion (solublizes too fast), and/or 2) low Ca availability (low solubility at 3<sup>rd</sup> timepoint), and/or 3) low total Ca, which means that more scrutiny is needed when feeding those limestones.

Regarding coarse limestone, average and median GMD was 1883 and 1906  $\mu$ m, respectively. More than 50% of the samples received were at or above 1900  $\mu$ m, and 10% were above 2500  $\mu$ m. Coarse limestone solubilized much slower than fine limestone, thus a longer incubation was applied to determine the solubility profiles of coarse limestone. At the first timepoint, the average solubility was 63%, but there was about 10% of the samples had >85%

solubility, indicating perhaps those limestones were not suitable to be used as "grit" for laying hens.

It is worthwhile to note that despite the generally accepted practice to relate limestone solubility to its particle size or GMD, our data have suggested strongly that the correlation is, if not very, weak. The particle size (GMD) only explained 35 and 11% of the solubility, respectively, for fine and coarse limestone in this project.

### Research progress to link limestone solubility to Ca, P digestion and animal performance

In 2019, Kim et al. (2019), published a study aiming to correlate the *in vitro* limestone solubility and Ca and P digestibility. The study suggested that limestone solubility and GMD are highly impactful on both Ca and P digestibility, in the presence and absence of phytase. Equations were published to predict the digestibility of Ca and (potential impact on) P, with a note from the authors to acknowledge the limitation of limestone samples used in the study and call for cautions in application. Taylor et al. (2019) used a titration approach to determine the extent of impact from limestone solubility and GMD on P digestibility with different phytase doses. They found that the high soluble limestone's impact on P digestibility persisted at 1000 FTU, but the impact was off set at 2000 FTU level. This suggested that P digestibility or release of P from phytate may not be optimized with high soluble limestone. But it was also suggesting this greater impact from highly soluble limestone can be managed with increasing phytase dose.

Research continued to answer the question on how this impact on digestibility is or is not linked to performance in birds. Venter et al., (2022) found birds fed high soluble limestone lost about 7 g BW, 1 point of FCR and 16 g of tibia ash at 16 d of age. At 32 d of age, BW and FCR was 23 g lower, 8 points higher compared to those fed slow soluble limestone (Soko et al., 2022). Similarly, Li et al. (2022) reported 70-190 g BW loss on end BW at 35 d. Li et al. (2022)

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also noticed that the limestone solubility related performance reduction was more severe in lower phytase group (500 FTU) vs. higher dose group (1500).

#### **Implication for applications**

As we are still making progress in understanding the variability of limestone and its bioavailability, and impact on phytase efficacy, animal performance and sustainability, it becomes clearer that limestone, being the cheap ingredient, is not cheap. Poor limestone bioavailability costs the animal on growth/performance which in turn drives the producer to formulate with more nutrients (inorganic P for example) to achieve growth targets. Due to various practical reasons, nutritionists' control over limestone and its quality is often limited thus it can be challenging to track accurately the limestone properties for feed formulation. Current evidence, though, does support using higher dose to overcome additional impact from those highly soluble limestones. Could this be something valuable for application? Maybe, if it costs \$0.30 for one unit inorganic P. But to do it properly, one should not forget that the substrate level (phytate), total limestone Ca (as feed, carrier, flow agent, filler, et al.), and intrinsic efficacy of the phytase product used.

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