MINERAL Writes

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Calcium content, or solubility, what has the greatest influence on bioavailability and digestibility.

Limestone is the fifth most abundant mineral in the Earth's crust, accounting for approximately 3.64% of its composition. The geological processes and locations that lead to limestone deposits can influence their calcium (Ca) content. The purity of limestone is determined by the percentage of calcium carbonate ($CaCO^3$) it contains. This raises the question: does the geographical location where limestone is mined affect its digestibility and bioavailability? Or are factors like particle size and solubility better indicators of digestibility and bioavailability? Perhaps it is all the above. This article will review a recent study that examined five sources of limestone, ranging in particle size and Ca percent, and solubility to determine the Ca digestibility and bioavailability using commercial broilers and crossbred chickens.

Higher purity of limestone leads to a greater concentration of $CaCO^3$ available for absorption. $CaCO^3$ has a 95-100% bioavailability, while ground limestone has a 90-95% Ca bioavailability. In the animal feed industry, $CaCO^3$, a Ca source, is defined by the Association of American Feed Control Officials (AAFCO) as having a minimum of 38% Ca. In comparison, ground limestone has a minimum of 33 % Ca.

Solubility can affect bioavailability. Solubility is the amount of Ca^{2+} ions that can dissolve in a specific volume of digestive liquid, such as hydrochloric acid in the proventriculus of chickens. The dissolution of $CaCO^3$ releases calcium for absorption, enabling the body to utilize it for various essential functions necessary for life. The following chemical reaction summarizes this process:

$CaCO_3 + 2H^+$ -------> $Ca^{2+} + H_2O + CO_2$ Stomach Reaction
$CaCl_2 + 2NaHCO_3 \rightarrow CaCO_3 + 2NaCl + CO_2 + H_2O$
Small Intestine Reaction $\rightarrow$ Absorption

Larger particles with lower in vitro solubility could lead to higher in vivo bioavailability in chickens due to extended retention and sustained dissolution in the gizzard's acidic environment. However, it's important to consider the specific characteristics of the particles. Large particles generally have a micron size of 3000 – 2000 microns, while fine particles are 500 microns or less.

Ca bioavailability can also be assessed by various bone parameters, including bone ash, weight, volume, breaking strength, and density.

Ca digestibility can be evaluated using several methods, including apparent ileal digestibility (AID), true ileal digestibility (TID), and standardized ileal digestibility (SID). Apparent digestibility does not account for endogenous losses and can underestimate the true digestibility. Standardized ileal digestibility corrects for basal endogenous losses and is considered more reliable. True ileal digestibility corrects for basal and specific endogenous losses and is the most accurate estimate of actual digestibility. There has been a move to formulate poultry diets based on digestible Ca rather than total Ca.

The review of a recent study, titled "Determination of calcium digestibility and bioavailability in 5 limestone sources using commercial broiler and crossbred chickens" (Drysdale et al., 2024), examined both male and female crossbred chickens, as well as male commercial broiler chickens. The research was conducted on chickens aged 9 to 22 days to assess the digestibility and bioavailability of Ca from five different limestone sources.

This study was set up in three experiments to determine the dietary Ca levels needed to achieve a linear increase in tibia bone ash in both crossbred and modern commercial broiler chickens. Compare Ca availability estimates for limestones obtained from two different bioassays. And evaluate the Ca availability in five commercial limestones that varied in solubility (88-97%), particle size, and geographical origin of limestone.

The first experiment was designed to measure the effect of dietary Ca levels on tibia bone ash to develop a slope-ratio Ca bioavailability in both male and female crossbred chickens and male commercial broiler chickens. Six dietary treatments were fed from 9 days of age to 22 days of age. Ca levels ranged from 0.20%, which was used as the Ca deficient diet, and increased by 0.15% for diets 2-6 with up to 0.95% supplementation.

The second experiment was conducted to determine the bioavailability of Ca in five limestones relative to Ca in reagent-grade $CaCO^3$ using bone ash as the primary response criterion. Thirteen diets were fed to commercial Ross 308 male broilers, which included a Ca deficient diet (0.30%) and diets supplemented with 0.15% or 0.30% Ca sourced from either reagent-grade $CaCO^3$ or diets supplemented with 0.15% or 0.30% from one of the five commercial limestones for a total of thirteen dietary treatments.

The third experiment aimed to determine the apparent ileal digestibility (AID) and total tract retention (TRR) of Ca using the five different types of limestone in broiler chickens. Experimental diets were fed from 18 days to 21 days of age.

Results from the first experiment showed that there was a quadratic response to increasing dietary Ca. Commercial broiler chickens showed significantly better body weight gain, feed intake, and feed efficiency than crossbred chickens. There was a quadratic response to increasing dietary Ca and bone ash content. These results show that a slope-ratio bone ash assay can measure the relative bioavailability of Ca in limestone. There was no significant interaction between dietary calcium levels and chicken types regarding growth performance, or between chicken types regarding bone ash content and concentration.

In the second experiment, weight gain, feed intake, and gain-to-feed ratios significantly increased with the inclusion of reagent-grade $CaCO^3$, and the test limestone sources compared to the calcium deficient diet. Bone ash content and bone ash concentration also showed improvement. There were no significant differences in performance among individual limestone sources. There was an increase in body weight gain for chickens fed the 0.30% supplemental calcium from reagent grade $CaCO^3$ compared to those fed the same calcium level from limestones L1, L2, or L4. Likewise, the supplemented reagent grade $CaCO^3$ had a higher gain-to-feed ratio fed at the same level as diets containing L1 through L4 limestone. Linear regression showed a strong positive relationship between supplemental Ca intake from all limestones and tibia bone ash. The relative bioavailability of Ca in the test limestones compared to reagent grade $CaCO^3$ was high (around 88-106%) and not significantly different among the limestone sources. This resulted in bioavailable Ca concentrations close to the analyzed levels in the limestones.

5 different limestone sources			
Limestone Sources	**Ca%**	**Mean Particle Size microns**	**Solubility %**
L1	39.3	516	97
L2	36.7	590	88
L3	38.2	652	95
L4	38.7	507	97
L5	37.9	714	93

Relative Ca bioavailability in the test limestone sources in Experiment 2					
	Total Ca in limestone (%)	**Bioavailability values[2] (%)**		**Bioavailable content[3] (%)**	
Limestone source		**Tibia ash (mg/tibia)**	**Tibia ash (%)**	**Tibia ash (mg/tibia)**	**Tibia ash (%)**
RCaCO3	-	100	100	-	-
1	39.3	93.3	92.4	36.7	36.3
2	36.7	96.3	88.2	35.3	32.4
3	38.2	98.8	98.7	37.7	37.7
4	38.7	89.8	89.5	34.8	34.6
5	37.9	106.2	96.6	40.4	36.7

[1]Abbreviations: $RCaCO_3$ = reagent grade calcium carbonate.
[2]Calculated by the slope-ratio method using the regression equation in footnotes 3 and 4 in Table 5 located in the published trial. .
Bioavailability values are relative to the Ca in reagent grade CaCO3 which was set at 100%. There were no significant differences ($P > 0.05$) from 100 for limestones 1-5 within columns.
[3]Bioavailable content = (Total Ca x bioavailability value)/100. Values are presented on as-fed basis.

In Experiment 3, the observed apparent ileal digestibility ranged between 21.5% and 34.3%, with no statistically significant differences identified among chickens fed the various sources of limestone. On the other hand, total track Ca retention exhibited significant variability, presenting values from 15.6% to 38.6%. Limestone from L3 and L4 had the highest TTR compared to the other sources of limestone. L5 had the highest AD and relative bioavailability. AID values were higher than TTR values, potentially due to urinary excretion of Ca. The differences in AID and TTR suggest that factors such as mine location, geology, and physical and chemical characteristics may impact it.

The study revealed variations in Ca digestibility and bioavailability among different sources of limestone, and that they can be influenced by factors such as Ca content, particle size, and solubility. To what extent of geographical location will be dependent of purity.

Based on research from (Kim et. al, 2019), it has been reported that 40% of Ca digestibility can be explained by particle size.

The study showed that by increasing Ca supplementation, a slope ratio could be used to measure relative bioavailability. However, further research is needed to assess the digestibility and total tract retention of Ca. Similar studies regarding Ca bioavailability have been conducted in laying hens, and it has been noted that particle size and the percentage of Ca content in limestone can influence production parameters, bone health, and eggshell quality. Further investigation into the role of Ca is essential due to the complexities associated with its functions in both broilers and laying hens.

Statistical discussion and explanation of the charts can be found in the article listed in the reference section along with additional charts.
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Growth performance, apparent ileal calcium digestibility, and apparent total tract calcium retention values for ad libitum-fed chickens in Experiment 3.[1,2]

Limestone source[3]	BW gain (g/chick)	Feed intake (g/chick)	Gain:feed (g/kg)	AID of Ca (%)	TTR of Ca (%)
1	67.8	243.9[ab]	278.0	20.4	19.2[b]
2	67.9	258.3[ab]	262.8	21.5	15.6[b]
3	67.1	240.5[b]	279.4	29.3	38.6[a]
4	70.3	261.0[a]	270.0	33.5	30.9[a]
5	67.8	249.0[ab]	272.6	34.3	20.4[b]
Pooled SEM	2.5	6.6	8.1	4.94	3.23

a-b Means within a column with no common superscript differ($P < 0.05$). 1Abbreviations: AID=apparent ileal digestibility; TTR=apparent total tract retention.
2 Values are means of 8 pens of 5 chickens at 22 d of age.
3 Corn-based diets calculated to contain 0.8% Ca from the limestone sources

References

R.L. Drysdale, P.L. Utterback, B.W. Parsons, and C.M. Parsons. 2024. Determination of calcium digestibility and bioavailability In 5 limestone sources using commercial broiler and crossbred chickens. Poult. Sci. 103:104226

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