



Implementation of a digestible calcium system: Why is it needed and where are we?

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SUMMARY

The regulation of blood calcium (Ca) and phosphorus (P) levels are closely intertwined, requiring that their metabolically available supply in the diet be carefully balanced. The majority of the P in plant-based feed ingredients is stored as phytate, which is poorly digestible by broilers, while inorganic P sources also show significant variability in digestibility. For decades, poultry nutritionists have incorporated available P in diet formulation, recognizing that the digestibility of P in feedstuffs is highly variable. In contrast, poultry diets are still formulated on a total Ca (tCa) basis, overlooking the variable digestibility of Ca from different dietary sources. Incorrect assumptions regarding the digestibility of Ca can lead to Ca imbalances, which negatively affect both broiler health and overall performance. Furthermore, the influence of Ca and limestone on phytase efficacy, which plays a critical role in enhancing P availability by breaking down phytate, is highlighted. As the poultry industry moves towards implementing a digestible Ca (dCa) system, the development of accurate prediction equations for limestone digestibility becomes essential. Such an approach not only improves the precision of diet formulation but also enhances overall broiler performance by ensuring a more balanced and metabolically available supply of both Ca and P. Transitioning to a dCa system, in conjunction with phytase use, represents a critical step in optimizing nutrient efficiency and improving sustainability in modern poultry production.

Description of problem

Poultry nutritionists started using an available phosphorus (AvP) value for ingredients as well as for requirements in the 1950s (NRC, 1954). The move to implement available or digestible nutrient values continued with bioassays being introduced for metabolizable energy (Hill et al., 1960), followed by digestible amino acids with methodologies introduced by Sibbald (1979) with the 1994 NRC including digestibility values in its Nutrient Requirement of Poultry, 9th edition. Today most poultry nutritionists formulate poultry diets based on AvP or digestible P (dP) and digestible amino acids. On the other hand, Ca continues to be formulated as a total nutrient irrespective of its availability. Work by several research centers has shown the importance of understanding digestibility of Ca (Angel, 2013; Anwar et al., 2018;

David et al., 2019; Kim et al. 2019; Li et al., 2021; Venter et al., 2024) highlighting large differences in digestibility between feedstuffs, especially between limestone sources.

Changing the way industry formulates for Ca from a tCa to a dCa system requires robust ingredient digestibility values, dCa requirements, validations of the ingredient values in diets for *in vivo* diet dCa, comparisons with formulated values, and implementation in commercial type diets to determine impact on performance and economics vs the existing tCa system.

This symposium paper will address: a) the need for a dCa system based on Ca and P homeostatic control and the implications of not formulating with a dCa system in terms of possible deficiencies or excesses; b) a need for work in laying hens and potential implications of not moving to a dCa system; c) effect of Ca source on phytase efficacy in

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the context of a global limestone quality; d) nutritional implication of having excess Ca in the diet; e) limestone characteristics with focus on Latin America; f) determination of dCa and dP in feed ingredients and the validation of these values in a complete diet; and g) use of prediction equations to understand Ca and P digestibility, and their implementation into commercial broiler production on performance, economics and sustainability. The need and importance to select ingredient and requirement dCa values determined using the same methodology, for commercial application will be discussed.

Calcium and phosphorus metabolism and regulation in poultry

A detailed overview of Ca and P metabolism in poultry is beyond the scope of this section. However, the reader is referred to existing reviews (David et al., 2023b; Li et al., 2016; Proszkowiec-Weglarz and Angel, 2013a; Sinclair-Black et al., 2024, 2023; Wideman, 1987). The regulation of Ca and P homeostasis is closely inter-related, in large part due to the deposition of most of the body's Ca and P in the form of bone hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$). When plasma levels of Ca or phosphate are low, bone is resorbed to release the required minerals. As such, homeostasis requires the co-regulation of Ca and P. Regulation of these minerals is via two endocrine pathways, involving parathyroid hormone (PTH) and $1,25(\text{OH})_2$ -vitamin D_3 , and three target tissues – the intestine, the kidney, and the bone. Blood Ca homeostasis is regulated through alterations in absorption from the intestine, reabsorption from the kidney and mobilization from bone or reduced retention in growing birds. Blood phosphate is regulated primarily through alterations in the rate of reabsorption from the kidney, with a lesser involvement of the bone and intestine.

At low to adequate dietary levels relative to the requirement, Ca is absorbed from the intestine through a saturable, active transport, but at high dietary levels, it is absorbed through a non-saturable, passive, transcellular mechanism. A similar situation exists for P absorption.

At low levels of plasma Ca, the parathyroid glands release PTH, which acts on the bone to increase resorption of hydroxyapatite, increasing both plasma Ca and phosphate concentrations. Simultaneously, PTH decreases Ca excretion, but increases phosphate excretion from the kidney, thus preventing an elevation of plasma phosphate. At low levels of plasma Ca or P, renal 1α -hydroxylase activity is increased, and $25(\text{OH})$ -vitamin D_3 is converted to the active metabolite, $1,25(\text{OH})_2$ -vitamin D_3 . The active vitamin D metabolite acts on the intestine to increase absorption of both dietary Ca and P at the kidney to decrease urinary phosphate excretion, and at the bone to release both Ca and phosphate.

When dietary Ca exceeds the requirement, but P is adequate, paracellular absorption increases the plasma concentration of Ca. Production of PTH and renal 1α -hydroxylase activity are decreased. Active transport of Ca and P from the diet is decreased, bone resorption is decreased, and renal excretion of Ca is increased. However, renal excretion of phosphate is decreased in order to maintain plasma phosphate levels.

At low plasma phosphate concentrations, intestinal P absorption and renal reabsorption are upregulated by increasing $1,25(\text{OH})_2$ -vitamin D_3 , in addition to bone resorption. When plasma phosphate level is high, bone fibroblast growth factor 23 (FGF23) is activated to increase renal excretion of P. In mammals, FGF23 has an inhibitory effect on PTH and renal 1α -hydroxylase activity, further reducing plasma P.

Implications of mistaken assumptions on dietary digestible Ca supply

Digestibility of Ca has been determined in 35 limestone sources from around the world resulting in a wide range of Ca digestibility values for broilers, with minimum, average and maximum values of 27.4, 45.9 and 68.5 %, respectively (Angel et al., 2023). The implications of mistaken assumptions of Ca digestibility from limestone on actual supply can be shown through a feed formulation exercise. A typical corn-soy basal diet was formulated to meet the nutritional needs of broiler starter chicks

(placement to 240 g BW), including a standardized ileal dCa requirement of 0.55 % (Angel et al., 2016; 2022) and dP requirement of 0.53 % (Jacobs et al., 2025), and the average Ca digestibility of limestone, based on taking the average of 35 Ca digestibilities in limestone (45.9 %; Angel et al., 2023). Note that given the variation in Ca digestibility in limestone, simply taking an average digestibility is never correct, but is done here for demonstration purposes. The basal diets represent reasonable levels of corn and soy for the respective formulation exercises, and re-formulations are based simply on the exchange of limestone and variable ingredients to provide adequate dCa under the various scenarios. Although other nutrients and energy are (incorrectly) assumed to remain constant under the various scenarios, the purpose of the exercise is to illustrate how mistaken assumptions can lead to over- or under-formulation of dCa under practical conditions.

In the exercises, tCa values for corn, soybean meal, dicalcium phosphate and limestone were based on the National Animal Nutrition database (National Animal Nutrition Program, 2024); ingredient values for dCa and dP were obtained from Angel et al. (2016; 2022); Proszkowiec-Weglarz et al., 2013b; and Venter et al. (2022). When assuming a limestone with average Ca digestibility of 45.9 %, the resulting diet contained 0.925 % tCa and 0.55 % dCa, if the assumption was correct. However, actual dietary dCa could range from 0.510 % to 0.599 % (−7.3 to +8.9 % of the dCa requirement) if the limestone used to mix the diets was at the low or high range of Ca digestibility, respectively (Table 1). If the nutritionist formulated the diet assuming the highest limestone digestibility of 68.5 %, the diet would contain 0.853 % tCa and 0.55 % dCa if the assumption was correct, but only 0.490 % or 0.517 % dCa (−10.9 or −6.0 % of the dCa requirement) if the actual limestone was of low or average Ca digestibility, respectively. Conversely, if the nutritionist were to assume that the limestone was at the low range of digestibility (27.4 %), the diet would contain 1.071 % tCa and 0.55 % dCa, but 0.617 % or 0.699 % dCa if the limestone was actually of average or high digestibility, respectively. Therefore, the same broiler starter diet formulation could have a range in dCa of 10.9 % below, to 27.1 % above the dCa requirement, with the tCa content of the diet ranging from 0.853 to 1.071 %, depending on the assumptions made. If the diet was formulated on to meet the tCa requirement of 0.95 %, (data not shown), the diet would contain 0.454, 0.528, or 0.617 % dCa if the limestone were of low, average, or high Ca digestibility. Clearly, it is critical to know the actual Ca digestibility of the specific limestone being used in order to correctly formulate the diets.

Phytate and calcium interactions

Increasing dietary Ca has long been known to exert negative effects on P utilization and performance of broilers. The main mechanism whereby Ca antagonizes P availability is through the formation of insoluble Ca-phytate complexes that are refractory to hydrolysis by exogenous and endogenous phytase at high pH in the small intestine. This was elucidated in early reports by Waldroup et al. (1964) that showed that widening the ratio of Ca: P in the diet of broilers reduced the utilization of phytate P.

Subsequent work by Nelson et al. (1968) confirmed that the negative impact of a wide range of Ca ratios on P utilization depends on the form of P present, particularly when the diets contain phytate. Numerous subsequent studies over the past decades have confirmed these early observations of the deleterious effect of Ca on both phytate hydrolysis, P digestibility, bone mineralization, and live performance (Selle et al., 2009). The chemistry of phytate and its interactions with divalent cations at the pH profiles in the different segments of the digestive tract of poultry play an important role in our understanding of how Ca exerts such a profound effect on dietary phytate P utilization (Angel et al., 2002). Phytic acid has 12 replaceable protons or reactive sites of which six are strongly acidic (pK of 1.5 to 2.0); two weakly acidic (pK ~ 6.0), and four very weakly acidic (pK of 9.0 to 11.0) (Costello et al., 1976; Erdman, 1979). Calcium and phytic acid chelate regardless of pH (Graf

Table 1Impact of mistaken assumptions regarding calcium digestibility of limestone on actual dietary digestible calcium levels in broiler starter diets (placement to 240 g).^{1,2}

Ingredient	Diet		Total Ca	Ca digestion coefficient			Actual diet digestible Ca		
	Amount (g/100 g)	tCa content (g/100 g)		Low	Mean	High	Low (g/100 g)	Mean (g/100 g)	High
Using average of Ca digestibility of limestone ³									
Corn ⁴	53.610	0.02	0.011	-0.350			-0.004		
SBM ⁴	36.300	0.42	0.152	-0.500			-0.076		
DCP ⁴	2.594	21.00	0.545	-0.680			-0.370		
Limestone	0.570	38.00	0.217	0.274	0.459	0.685	0.059	0.099	0.148
Other	6.926	0	0	-0.000			-0.000		
Total	100.000		0.925				0.510	0.550	0.599
Variance from requirement (%)							-7.3	—	+8.9
Using high extreme of Ca digestibility of limestone ³									
Corn ⁴	53.610	0.02	0.011	-0.350			-0.004		
SBM ⁴	36.300	0.42	0.152	-0.500			-0.076		
DCP ⁴	2.594	21.00	0.545	-0.680			-0.370		
Limestone	0.383	38.00	0.146	0.274	0.459	0.685	0.040	0.067	0.100
Other	7.113	0	0	-0.000			-0.000		
Total	100.000		0.853				0.490	0.517	0.550
Variance from requirement (%)							-10.9	-6.0	—
Using low extreme of Ca digestibility of limestone ³									
Corn ⁴	53.610	0.02	0.010	-0.350			-0.004		
SBM ⁴	36.300	0.42	0.152	-0.500			-0.076		
DCP ⁴	2.594	21.00	0.545	-0.680			-0.370		
Limestone	0.955	38.00	0.363	0.274	0.459	0.685	0.099	0.167	0.249
Other	6.540	0	0	-0.000			-0.000		
Total	100.000		1.071				0.550	0.617	0.699
Variance from requirement (%)							—	+12.2	+27.1

¹ Digestible Ca requirement: 0.55 % (Angel et al., 2016; 2022); digestible P requirement: 0.53 % (Jacobs et al., 2025).² Total Ca values obtained from the National Animal Nutrition Database (NANP, 2024).³ Standardized ileal digestible Ca in various limestone sources (Angel et al., 2023).⁴ Ca digestibilities from Angel et al. (2016; 2022); Proszkowiec-Weglarz et al., 2013b; Venter et al. (2022).

1983; Tamim and Angel, 2003, Tamim et al., 2004). The degree of Ca-phytic acid chelation increases with increasing pH, with this chelation being dependent on the molar ratio of Ca: phytate in the small intestine (Wise, 1983; Martin and Evans 1986). Chelated Ca and phytic acid are soluble at a pH below 4.0 but as pH increases, the chelated phytate precipitates. Therefore, as the phytate moves distally along the gastrointestinal tract past the gastric area, and the pH of the digesta increases and precipitation of the phytate increases until it is complete at pH between 6.0 and 7.0 (Tamim et al., 2004). The insoluble or precipitated complex is inaccessible to hydrolysis by exogenous or endogenous phytase enzymes in the small intestine (Tamim and Angel, 2003; Selle et al., 2009).

The formation of Ca-phytate complexes and subsequent precipitation of Ca-phytate at the pH of the small intestine (> pH 5.5) results in reduced phytate hydrolysis by both endogenous and exogenous phytases, leading to reduced digestibility of phytate P. Potential interactions of phytate and divalent cations such as Ca at the acidic pH of the gastric stomach and impacts on phytate hydrolysis of exogenous phytase enzymes are less well understood. Graf (1983) suggested that phytate was able to chelate Ca at a pH as low as 2.0 with substantial amounts of cations potentially being bound to phytate in the acid stomach that may then facilitate the precipitation of the complex during the subsequent passage through the intestine. Tamim and Angel (2003) further elucidated the interaction of Ca-phytate at both low and high pH as being dependent on the molar ratio of Ca: phytate. At pH 6.5, molar ratios of Ca: phytate of 6.9: 1 and 15.5: 1 resulted in 45 and 57 % inhibition in phytate P hydrolysis, respectively, during a 30 min incubation period. At pH 2.5 however, 50 % inhibition in phytate P hydrolysis in 30 min incubation was observed only at when the Ca: phytate molar ratio was 15.5: 1. This understanding that the antagonistic effects of Ca on P utilization are initiated via the formation of Ca-phytate complexes that impair the efficacy of phytase at low pH in the proventriculus and gizzard has been fundamental to the current work of developing strategies to limit the impact of Ca on P utilization in broilers. In this context, the source of Ca and the rate at which free soluble Ca ions are

released at low pH in the proventriculus and gizzard is important in determining the extent of Ca-phytate formation in this segment of the digestive tract and subsequent impacts on phytase efficacy. Work by Li et al. (2021) has suggested that Ca from limestone that is rapidly solubilized in acid has a greater propensity to antagonize phytate P hydrolysis by exogenous phytase than the Ca from bone meal. This results from a slow release of Ca ions from the hydroxyapatite crystal in bone meal because the organic matrix in which the crystal sits must be digested before the crystal can solubilize in the proventriculus and gizzard as compared to Ca from limestone, thus leading to a slower rate of Ca-phytate complex formation, allowing greater hydrolysis from added phytase.

Limestone quality and phytase efficacy

Limestone quality encompasses the concentration of Ca and other minerals and heavy metals, as well as characteristics such as particle size, rate of solubilization in acid, the geology of the limestone rock and acid binding capacity of the limestone. Previous reports have described the large variation that exists in the trace mineral concentration of limestone, as well as its physical characteristics and dynamic solubility (Plumstead et al., 2020; Gilani et al., 2022). Since Ca-phytate complex formation at low pH has a large negative impact on exogenous phytase hydrolysis of phytate in the proventriculus and gizzard, variation in the rate of solubilization and subsequent release of free Ca ions (reactive Ca) between limestones can be expected to result in variation in the degree of Ca-phytate complex formation and subsequent phytase enzyme efficacy. Manangi and Coon (2007) showed a stepwise increment in P release at 15 min from an *E. coli* phytase *in vitro* at pH 2.5 when limestone particle size was increased from 28 µm to 519 µm with subsequent reductions in P release at greater limestone particle sizes. While not determined, these authors hypothesized that a larger particle, and consequently slower soluble limestone, could potentially be beneficial for phytase efficacy *in vivo*. Subsequent work has clearly demonstrated that the solubility kinetics of limestone could alter P digestibility and the

net increment in digestible P following phytase addition to diets (Kim et al., 2018, 2019; Li et al., 2021; Taylor et al., 2019). Instructively, Kim et al. (2018) showed an interaction between dietary Ca level and limestone particle size with incremental levels of Ca reducing in the efficacy of phytase in increasing digestible P. They demonstrated a stepwise negative effect as fine limestone inclusion was increased in the diet, but not when coarse limestone was used. This suggests that the negative effect of excess limestone on P digestibility and phytase efficacy could be mitigated by selecting a limestone with slower rate of solubility. In the same study, these authors also demonstrated an interaction between dietary Ca level and limestone particle size on apparent ileal digestibility (AID) of Ca. The AID of Ca was increased by 5.0 % when the diet contained 1.0 % Ca while it was increased by 15.0 % when the diet contained 0.6 % Ca when fine limestone was used as the Ca source, while the increase due to phytase inclusion was only seen in birds fed 1.0 % Ca with coarse limestone.

The molar ratio of Ca: phytate is pivotal in determining the extent of Ca: phytate complex formation and subsequent inhibition of hydrolysis of phytate by exogenous phytase enzymes. Therefore, the mechanism whereby limestone particle size alters P digestibility from phytase can be explained by the amount of solubilized ionized Ca (reactive Ca) release from the different limestones that increases the formation of Ca: phytic acid chelates. In addition to changing Ca concentration, the molar ratio of Ca: phytate can be altered by reducing the amount of phytate present in the proventriculus and gizzard through the addition of exogenous phytase to the feed. Differences exist between exogenous phytase products in terms of the pH optima of the enzymes, the affinity of the enzymes for phytate substrates, and the micrograms of phytase protein included commercially in 1 phytase unit (FTU). Since FTU are standardized at pH 5.5, differences between phytases in their pH optima and Michaelis-Menten kinetics of the phytase, will lead to large differences in enzyme activity at the gastric pH of the proventriculus and gizzard. For example, the seven commercial enzymes evaluated by Menezes-Blackburn et al. (2015) showed enzyme activity at pH 3.0 to range from 235 % to as low as 12.5 % relative to enzyme activity at pH 5.5 at which phytase FTU are determined. This suggests that different phytases will have varying propensity to rapidly degrade phytate in the proventriculus and gizzard, resulting in potential differences in the amount of phytate that can chelate Ca. Using one example, Taylor et al. (2019) evaluated the interaction of limestone particle size (0.123, 0.174, 0.722 mm geometric mean diameter (GMD)) and two sources of phytase (*E. coli* and *Buttiauxella* spp. phytase) included in diets at 1000 FTU/kg feed on ileal P digestibility in 21-day old broilers. There was an interaction ($P < 0.05$) between limestone particle size and phytase source, with the P digestibility from *E. coli* phytase being more adversely affected by limestone with smaller particle sizes and a faster rate of solubilization. From this work it can be hypothesized that the extent whereby limestone interacts with phytate in the proventriculus and gizzard and alters phytase efficacy is influenced by the interaction of how fast the limestone solubilizes to release free Ca and the rate at which phytases hydrolyze phytate. Increasing the dose of phytase enzymes and increasing the ratio of enzyme: substrate is a further tool whereby the rate of phytate hydrolysis can be increased. Recent work by K. Venter (unpublished data) that evaluated incremental doses of phytase (0, 1000, and 2000 FTU/kg feed) and limestones from four different sources with different solubilization rates showed an interaction ($P < 0.05$) between limestone source and phytase dose. At 0 and 1000 FTU added phytase, there was an effect of limestone source on ileal P and Ca digestibility that resulted in significant effects of limestone on the increment in P digested from adding 1000 FTU phytase. However, at 2000 FTU, differences between limestones on P digestibility were less evident, with lower differences in the increment of digestible P between 1000 and 2000 FTU phytase. This supports that increasing the rate of phytate hydrolysis by increasing the dose of phytase can reduce the deleterious effect of more rapidly soluble limestone on phytase enzyme efficacy.

Evaluation of limestone characteristics in Latin America

Carbonate rock makes up to 10 to 15 % of all sedimentary rocks. These rocks are classified as either Calcitic limestones (CaCO_3), or high Mg calcite - dolomitic limestone - which are mainly composed of equal moles of CaCO_3 and MgCO_3 . Magnesium calcite is a variant of calcite where Mg ions replace some of the Ca ions in the crystal structure. The general chemical formula for magnesium calcite can be represented as $(\text{Ca}_{1-x}\text{Mg}_x)\text{CO}_3$, where (x) typically ranges from 0.04 to 0.30, with x varying between 0.04 and 0.3 and a MgCO_3 percent of 7 to 11 %. While magnesium calcites have the gross crystallographic characteristics of calcite, magnesium substitution affects its physical structure and solubility (Kim et al., 2021).

A total of 123 samples of limestone were collected from the broiler industry in several Latin American countries (Argentina, Brazil, Colombia, Costa Rica, El Salvador, Guatemala, Honduras, and Mexico). All samples were analyzed for Ca content (inductively coupled emission spectroscopy, according to the AOAC method 985.01, AOAC, 1996), GMD (ANSI/ASAE method S319.4 (2008)), and dynamic solubility (Kim et al., 2019). This method uses a pH 3 glycine-buffered solution and solubility is determined at 42°C at 3 different time points depending on whether the sample is coarse or fine. Limestone with GMD higher than 800 μm were classified as coarse, and lower than 800 μm were classified as fine. Solubility of coarse limestones were analyzed at: 30, 90 and 150 min while solubility for fine limestones was determined at 5, 15 and 30 min.

Results, by country of limestone origin, for GMD and Ca are presented in Table 2 and dynamic solubility in Table 3. Variability within the fine limestones, both within country and overall, was high for GMD with the greatest GMD seen in one sample in Mexico (1006 μm) and lowest in Brazil (83 μm) (Table 2). Averages within country can be interpreted only in Brazil ($n = 79$), Mexico ($n = 15$) and Costa Rica ($n = 11$) because the number of samples analyzed for other countries was too low. Overall mean limestone GMD was low in all countries, with Brazil having the highest mean of 411 μm . Limestones of low GMD continue to be used, which with what we know today, are limestones with higher solubility at 5 min (Table 3) that is negatively correlated to phytase efficacy and P digestibility as well as affecting the digestibility of other mineral cations and amino acids. Concentrations of Ca in some limestones in every country where samples were taken are below (< 36 %) the minimum level of Ca that limestones should have (Table 2). On average, though, limestones in all countries were above 36 % Ca with some samples analyzing at 40 % Ca. Variability in solubility was high ($P < 0.05$) at 5 and 15 min with smaller differences at 30 min. Pearson correlations between GMD and 5, 15, 30 min solubility were -0.45 , -0.38 , and -0.26 (Table 4). The correlation between GMD and the various solubilities indicates that GMD has a clear impact on solubility; however, the moderately low R^2 value suggests that there are additional factors influencing solubility, such as rock morphology and chemical composition. The high correlation between 5-, 15-, and 30-min solubilities is to be expected with this method as 15 min solubility is impacted by 5 min solubility. Solubilities at times past 5 min cannot be lower than those at 5 min.

Calcium digestibility for feed ingredients

Limestone is the primary raw material contributing Ca in broiler diets, accounting for over 65 % of the total analyzable Ca in most diets. Accurate quantification of Ca digestibility from limestone is therefore crucial in the transition towards a dCa system. Data on limestone Ca digestibility has been published primarily by two research groups (Angel, 2013; Anwar et al., 2015, 2016a, b; Kim et al., 2018, 2019; Li et al., 2021; Angel et al., 2023; Venter et al., 2024). Data on Ca digestibility for most ingredients of plant origin is available, but in most cases consists of only 1 sample, thus making this data is far from robust. Generally, plant-based feed ingredients have a lower Ca content

Table 2
Limestone geometric mean diameter (GMD) and Ca content by Latin American country of origin.

Origin	n	GMD ¹ , μm				Ca ² , %			
		Mean	Min	Max	SE	Mean	Min	Max	SE
Argentina	6	371	99	686	107	36.7 ^{bc}	32.6	39.3	1
Brazil	79	411	82	895	25	38.3 ^a	33.0	40.3	0.2
Colombia	3	378	165	742	183	36.6 ^{cb}	33.5	38.7	1.6
Costa Rica	11	359	172	587	43	37.7 ^{ab}	34.5	39.7	0.5
El Salvador	4	379	122	663	146	36.9 ^{abc}	34.8	38.3	0.7
Guatemala	1	165				36.5 ^{bc}			
Honduras	3	186	155	208	16	35.0 ^c	33.3	37.7	1.6
Mexico	15	409	174	1006	60	36.8 ^{bc}	35.6	38.8	0.7
P-value		0.69				0.001			

¹ Geometric mean diameter, ANSI/ASAE method S319.4 (2008).

² Ca determined using ICP using 35.13 AOAC method (AOAC, 1999).

Table 3
Limestone dynamic solubility by Latin American country of origin.

Origin	n	5 min solubility ¹ , %				15 min solubility, %				30 min solubility, %			
		Mean	Min	Max	SE	Mean	Min	Max	SE	Mean	Min	Max	SE
Argentina	6	64 ^{ab}	48	90	6.2	84 ^{ab}	69	99	4.5	91	78	99	3.4
Brazil	79	55 ^b	24	88	1.6	75 ^{bc}	45	94	1.2	87	67	97	0.8
Colombia	3	47 ^b	17	69	15.9	68 ^c	46	81	11.1	83	74	93	5.3
Costa Rica	11	62 ^b	35	86	5.3	83 ^{ab}	59	96	4.2	91	76	99	2.6
El Salvador	4	84 ^a	77	89	2.5	90 ^a	88	93	1.0	91	88	92	0.8
Guatemala	1	81 ^a				93 ^a				98			
Honduras	3	73 ^{ab}	69	80	3.5	83 ^{ab}	79	96	2.3	88	83	93	3.0
Mexico	15	77 ^a	32	93	4.2	84 ^a	61	95	2.6	91	76	97	1.6
P-value		< 0.01				0.02				0.13			

¹ Determined following the method of Kim et al. (2019).

Table 4
Pearsons correlations between limestone characteristics.

	GMD ¹ , μm	5 min solubility, %	15 min solubility, %	30 min solubility, %	Ca, %
GMD, μm	1.00	-0.45	-0.38	-0.26	0.20
5 min solubility, %	-0.45	1.00	0.88	0.67	-0.25
15 min solubility, %	-0.38	0.88	1.00	0.87	-0.14
30 min solubility, %	-0.26	0.67	0.87	1.00	0.03
Ca content, %	0.20	-0.25	-0.14	0.03	1.00
P-Values					
GMD, μm	-	0.001	0.001	0.010	0.641
5 min solubility, %	0.001	-	0.001	0.001	0.003
15 min solubility, %	0.001	0.001	-	0.001	0.299
30 min solubility, %	0.010	0.001	0.001	-	0.939
Ca content, %	0.641	0.004	0.299	0.939	-

¹ Geometric mean diameter, ANSI/ASAE method S319.4 (2008).

compared to those of animal origin, making it challenging to determine Ca digestibility, particularly for grains that in general contain very low concentrations of Ca (i.e., corn contains 0.1 to 0.3 % Ca). When assessing Ca digestibility coefficients for feed ingredients with low Ca content, it is essential to develop a repeatable methodology that considers homeostatic changes if diets are not balanced or are deficient or in excess in Ca and P. Regardless of the low concentration of Ca in grains, it is important

to develop a robust database for grain dCa.

In a typical commercial starter diet with an inclusion of 35 % soybean meal with a Ca content of 0.24 %, soybean meal can contribute 0.08 % tCa or around 12 % of the analyzable Ca in the diet. Robust dCa values for soybean meal and other plant-based protein sources as well as grain or grain by-products are needed to correctly implement a dCa system. Feed ingredients that contribute significant amounts of Ca to the diet, such as meat and bone meal, poultry by-product, monocalcium phosphate, and dicalcium phosphate, have been evaluated (Angel, 2016; Angel et al., 2016; Anwar et al., 2016c; Li et al., 2021). Animal by-product proteins are particularly variable in terms of Ca, P, and protein content, depending on the origin and the proportion of soft tissue and bone in the final product. Values for Ca digestibility in high Ca content ingredients vary greatly between research groups (Angel, 2016; Anwar et al., 2016c). An example of this is when comparing the SID Ca values for meat and bone meal from Angel (2016) and Anwar et al. (2016c), values range between 50 and 65 % and 41 to 56 %, respectively. This variability may be due to differences in the inherent content of Ca in the ingredient as well as fundamental methodology differences, as outlined by Angel et al. (2022).

Important methodological differences include the duration of the study, and the basal diet used, age of the broilers in relation to Ca concentration used, the level of Ca in the test diet and in the diet that broilers are fed prior to the start of the trial. Research by Proszko-wiec-Węglarz (2013b) noted that when broilers were fed the test diet for 32 to 38 h, the absorption capacity established by the "normal commercial" diet was maintained, preventing measurable changes in distal ileal digestibility as part of the body's homeostatic mechanism for Ca. Instructively, when test diets were fed for longer than 32 to 38 h digestibility of Ca changed as a result of the bird's homeostatic mechanisms. Differences in methodology between research groups in the time test diets are offered prior to determination of Ca digestibility will therefore contribute to differences in reported dCa values of ingredients.

By combining existing values determined using a standard methodology (Angel et al., 2022; Venter et al., 2022; 2023), dCa values are now

available for most feed ingredients commonly used in broiler diets (Table 5).

Validating existing values of digestible Ca in a complete diet

When developing a new system like dCa, it is necessary to validate this for accuracy and additivity. Venter et al. (2023) conducted a study to validate the additivity of dCa of individual dCa values for various feed ingredients from Table 5. Typical commercial broiler grower diets were formulated using dCa values for ingredients and the dCa of the complete feed determined *in vivo* and compared to dCa values formulated based on ingredient dCa values. Two studies were done to validate ingredient (corn, soybean meal, meat and bone meal, full-fat soybean meal, sunflower meal, rapeseed meal, rice bran, and wheat bran) dCa values. Results demonstrated that the *in vivo* determined dCa values closely aligned with the formulated dCa values, with only minor deviations observed. For instance, in feed formulations containing corn, wheat, soybean meal, full-fat soybean meal, rapeseed meal, and sunflower meal, the formulated dCa value was 0.358 %, while the *in vivo* determined value was 0.362 %, indicating additivity as well as accuracy. Similarly, for feed with corn, soybean meal, and full fat soybean meal, the formulated dCa was 0.348 %, and the *in vivo* determined value was

Table 5
Standardized ileal digestibility (SID)¹ of calcium of feed ingredients from different research groups.

Ingredient	Massey University		University of Maryland (UMD)/ University of Pretoria (UP)	
	SID Ca, %	Reference	SID Ca, %	Reference
Corn			38 Min: 20 Max: 46 n = 16	Angel et al., 2016, 2022; Proszkowiec-Weglarz et al., 2013b; Venter et al., 2022.
Soybean meal ²	53 CP: 45.3 % Ca: 0.29 % P: 0.63 % n ³ = 1	David et al., 2021a	49 Min: 47 Max: 65 n = 14	Angel et al., 2016, 2022; Proszkowiec-Weglarz et al., 2013b; Venter et al., 2022.
MCP	38 Min: 33 Max: 45 n = 3	Anwar et al., 2018; David et al., 2019	74 Min: 69 Max: 77 n = 9	Angel et al., 2016, Angel et al., 2022; Proszkowiec-Weglarz et al., 2013b
DCP	31 Min: 28 Max: 36 n = 3	Anwar et al., 2018; David et al., 2019	68 Min: 64 Max: 72 n = 11	Angel et al., 2016, Angel et al., 2022
Meat and bone meal	49 Min: 41 Max: 56 n = 4	Anwar et al., 2016c.	60 Min: 50 Max: 65 n = 12	Angel et al., 2016

¹ Only SID Ca values are being reported in this table.

² 48 % soybean meal (range 46.2-48 %) unless specified otherwise.

³ n refers to the number of samples of an ingredient where either apparent or standardized digestible Ca have been done.

slightly lower at 0.336 %.

The minimal variation between formulated and *in vivo* determined dCa values across all feed formulations suggests that the formulation process for dCa in commercial type diets with different ingredients is highly accurate in predicting the actual dCa content for broilers.

Implementation of a digestible Ca system and subsequent impact on performance

One hurdle in transitioning to and implementing a dCa system has been the limited research on its application in broiler diets, including a limited understanding the optimal dCa requirements for performance and skeletal health. This has driven the recent concerted effort to develop dCa requirements for broiler chickens (Walk et al., 2021, 2022a, b, 2024; David et al., 2021, 2022, 2023a; Angel et al., 2023; Venter et al., 2023). A series of studies (Jimenez-Moreno et al., 2013a, b; K. Jacobs, University of Pretoria, South Africa, personal communication) was also conducted with the use of corn-soybean meal-based diets to determine the dCa and dP requirements for broilers (Angel et al., 2022). Discrepancies noted between authors (Table 6) as to dCa requirements values and dCa to dP ratios can be in large part explained by the methodology used in the studies and especially based on the methodology used to determine the feed ingredients' dCa values used in formulating the diets used to determine requirements. It is critical that when formulating diets using dCa requirements, the dCa ingredient values used in diet formulation are defined and from a consistent source.

The use of prediction equations to understand Ca and P digestibility in limestones

Two main research groups have determined Ca digestibility of limestone in broilers and have shown that this can vary dramatically depending on the Ca source provided, the solubility and source (geology) of limestone, as well as by the diet source and level of phytate and supplemental level of phytase in the diet (Anwar et al., 2016c; Angel, 2019; Kim et al., 2019; Taylor et al., 2019; Angel et al., 2023). In current commercial diets where high phytase doses are used, limestone becomes the primary contributor of Ca in the diet, accounting for over 65 % of the total analyzable Ca. Due to the significance of limestone as a contributor of dietary Ca, understanding limestone Ca digestibility, and limestone Ca interactions with phytate becomes essential if a dCa system is to be effectively implemented.

Limestone particle size (Anwar et al., 2016b; Kim et al., 2018, 2019) and solubility as well as geology (Kim et al., 2019) can affect the utilization of limestone Ca by broilers. Due to the variable nature of limestone and the dCa from it being so variable (Anwar et al., 2016b; Kim et al., 2019), it became essential to develop equations that would accurately predict the dCa in limestone based on *in vitro* assays.

Anwar et al. (2016b) and Kim et al. (2019) demonstrated that a positive correlation exists between limestone particle size and *in-vivo* Ca digestibility, with Ca digestibility being negatively correlated to limestone particle size (Anwar et al., 2016b; Kim et al., 2018) and solubility (Kim et al., 2019). Further publications by the same group, Angel (2019), and Kim (2019) have subsequently provided greater insight into the impact that differences in limestone solubility arising from various sources (geology of limestone), or different particle size, can have on Ca digestibility in broilers. When comparing limestone from the same source, a reduction in particle size from 800 µm GMD to 150 µm GMD reduced standardized ileal digestibility (SID) of Ca from 49 % to 38 % (Li et al., 2021). Importantly, in the paper by Kim (2019), differences in GMD particle size alone could only explain < 40 % of the observed differences in Ca digestibility from limestone; differences in limestone geology and physical/chemical characteristics were equally important to particle size in their potential effects on Ca digestibility.

Extensive work has been done (Angel et al., 2023) on developing an equation that would allow feed mills to analyse limestone batches in

Table 6
Broiler pre-starter/starter requirements.

Body weight at end of first feeding phase, g	Walk et al. (2024)		David et al. (2021)		Angel et al. (2023)		Jacobs et al. (2025)	
	Bone ash, %	BWG ¹ , g	Bone ash, %	BWG, g	Bone ash, %	BWG, g	Bone ash, %	BWG, g
dCa ²	0.53	-	0.45	0.32	0.55	-	0.55	-
Phosphorus	0.48 ²	-	0.50 ³	0.50 ³	0.53 ⁴	-	0.53 ⁵	-
dCa: AvP or dP	1.1:1	-	0.90:1	0.64:1	1.04:1	-	1.02:1	-

¹ Body weight gain.

² Coefficients for available P (pre-cecal P) from Shastak and Rodehutsord, 2015 were used to formulate the experimental diets but the dCa requirement estimate was based on dCa determined in the experimental diets.

³ Digestible P (dP) values used to formulate the diets were from Mutucumarana et al., 2015; Van Harn et al., 2017; Shastak et al., 2012.

⁴ Digestible P determined for all treatments in the experiment and where requirements were determined that value of dP was used (Jimenez-Moreno et al., 2013a).

⁵ Digestible P values used to formulate the diets were from Angel et al., 2022; Venter et al., 2022.

in vitro and predict a dCa value for the specific limestone that can be used in diet formulations. Prediction equations for dCa in limestone based on 35 limestones have now been developed that include seven factors: solubility at 5, 15, and 30 min; GMD; Ca and Mg content in the limestone and phytate P concentration in the diet the limestone is being added to. The equations have been validated using limestone samples not used in the development of the prediction equation. The correlations between predicted and actual *in vivo* dCa values determined in trials were r^2 0.93 and root square mean error (RMSE) 2.56 and r^2 0.96 and RMSE of 2.75 in diets without and with phytase supplementation, respectively (Angel et al., 2023).

Impact of implementing a dCa system on performance, economics and sustainability

Two broiler performance studies were conducted in 2023, applying the dCa formulation system in a Latin American context and in South Africa. In the Latin American study, done at an integrator's research farm, one low solubility limestone (22, 60, 91 % solubility at 5, 15 and 30 min based on the Kim et al. (2019) method and with a predicted dCa of 64.5 % was used and corn/soybean meal/ full fat soy-based diets were formulated to two Ca and P systems (R. Angel, unpublished data). The two systems tested were a tCa/dP and a dCa/dP system to allow for a determination of whether the dCa system implementation would result in performance and economic improvements. Male broilers fed the diets formulated to dCa/dP weighed more ($P < 0.001$) vs those fed diets formulated to tCa/dP at 37 d of age, 2748.1 and 2703.6 g, respectively. Feed conversion (feed intake; gain) was also lower ($P < 0.001$) for the whole growth period to 37 d of age, when broilers were fed the dCa/dP diets (1.419) as compared to broilers fed the tCa/dP diets (1.457). Feed costs (September 6, 2024, prices) of production in US dollar cents per kg of gain to 37 days were also lower ($P < 0.001$) when broilers were fed the dCa/dP diet as compared to those fed the tCa/dP diets. Similarly, in a trial done in a research center in South Africa (Neuro Livestock Research, Kameeldrift, Brits, South Africa; K. Venter, unpublished data) in 2023, broilers that were fed diet formulated to dCa/dP grew faster ($P < 0.01$) than those fed diets formulated to tCa/AvP with BW at 32 d of age of 1945.1 and 1919.9, respectively. Feed to gain ratio was also reduced ($P < 0.01$) when diets formulated to dCa/dP were fed (1.442) as compared to that of broilers fed tCa/AvP formulated diets (1.482). Feed costs, based on Brazilian September 6, 2024, prices (US dollars cents) per kg of broiler produced to 32 d of age was lower ($P < 0.01$) when dCa/dP formulated diets were fed (41.74) as compared to when tCa/AvP diets were fed (44.77).

Conclusions and applications

1. To optimize broiler performance and health, a supply of Ca at tissue level that is close to requirements for different metabolic functions and physiological processes is required. Given the vast potential

range in actual Ca digestibility of limestone from different sources, and the current limited knowledge of broiler and laying dCa requirements, it is possible for commercial poultry diets formulated to tCa to contain substantial excess or deficiency of dCa.

2. To prevent such dietary imbalances, it is important to have a confident assessment of the dCa content of each limestone being sourced, as well as the dCa requirements of the birds being fed.
3. Although there has been progress towards understanding these concepts in broiler chickens, similar data is almost entirely lacking for laying hens.
4. Transitioning to dCa-based formulations will enable nutritionists to more precisely adjust the Ca content in broiler diets to reflect Ca digestibility in various ingredients and thereby provide diets that supply Ca at tissue level closer to the bird's requirement. Choice of limestone has a significant impact on phytate hydrolysis from exogenous phytase and greatly impacts the phytase matrix for dCa and dP that can be applied.
5. Prediction models that can describe the impact of different limestones on dCa and dP from phytase will allow more precise delivery of both dCa and dP required by the bird, nutritionists to formulate closer to requirements, thus reducing excess in diet Ca and P in commercial broiler diets and reduce dependence on supplemental inorganic P. This reduction is crucial from a sustainability perspective.

Declaration of conflict of interest

The authors declare that they have no known conflicts of interest, financial or otherwise, that could have influenced the outcomes of this study or its interpretation.

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