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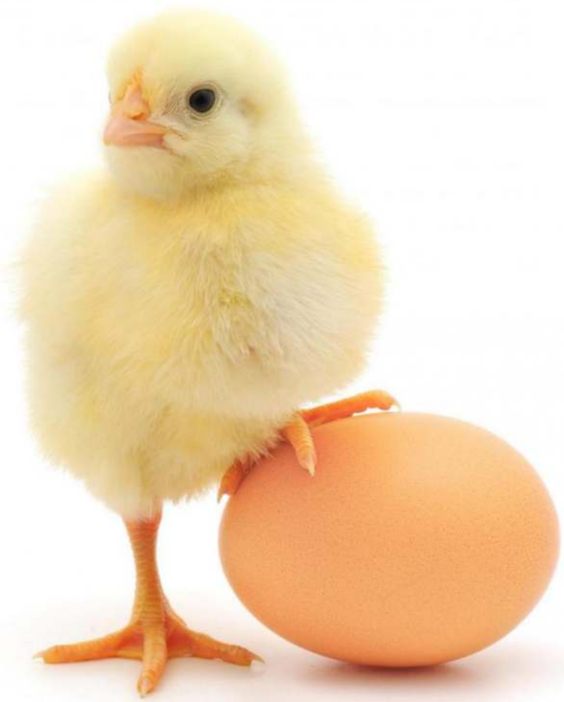

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The Effect of Calcium Carbonate Particle Size and Solubility on the Utilization of Phosphorus from Phytase for Broilers

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Abstract: A twenty-eight day floor pen experiment was carried out using 1680 Cobb-500 male day old chicks to evaluate the impact of feeding different particle sizes of calcium carbonate ($CaCO_3$) on broiler performance and tibia ash. The experiment consisted of 8 treatments with 6 replications per treatment to test 8 different $CaCO_3$ particle sizes. The corn-soybean meal based diets contained 21.5% CP, 3025 kcal ME kg^{-1}, 0.78% Ca, 0.20% Non-phytate P (NPP) with 500 FTU kg^{-1} of Danisco Phyzyme XP added. The average particle sizes of $CaCO_3$ (along with % solubility) tested were 28 (74.4), 137 (56.4), 299 (47.0), 388 (53.0), 519 (46.7), 760 (45.3), 796 (42.2) and 1306 (43.4) µm. Significantly ($p<0.05$) increased weight gains were obtained for chicks fed $CaCO_3$ particle sizes between 137 and 388 µm compared to the gains obtained by feeding either the smallest (28 µm) or largest particle (1306 µm) sizes. An increased mg of ash per tibia was also obtained for the chicks fed $CaCO_3$ particle sizes ranging from 137-388 µm as compared to the smallest (28 µm) or largest particle (1306 µm) sizes. An *in vitro* phytate P (PP) hydrolysis by a 3-phytase at pH 2.5 and 6.5 using the same 8 different particles sizes of $CaCO_3$ at equivalent to 9 g kg^{-1} diet was carried out to evaluate the effect of Ca particle size on PP hydrolysis at 15, 30, 60 and 120 min incubation at 37°C. The results indicate a significant ($p<0.05$) interaction of Ca particle size and pH on PP hydrolysis with greater effect at pH 6.5. The main effect of particle size showed that the smallest particle size (28 µm) with more solubility (74.4%) had the lowest PP hydrolysis indicating the interference on the action of phytase on PP hydrolysis due to Ca-phytate complex formation. In summary, both *in vivo* and *in vitro* studies indicate that a small Ca particle size (28 µm) $CaCO_3$ with a high solubility (>70.0%) limits PP hydrolysis to provide available P for growth and bone ash formation.

Key words: $CaCO_3$ particle size, phytate hydrolysis, broiler performance

Introduction

There is considerable research that has been reported describing the benefits of feeding large particle calcium carbonate ($CaCO_3$) to layers and breeders. The large particles will increase the shell quality and also improve the bone ash and bone strength of layers. The laying birds gain a benefit from the large particle because the particle stays in the gizzard during the non-feeding periods and contributes Ca to the laying bird during the time period when egg shell is being made. The amount of research that has been conducted to test different particle sizes of $CaCO_3$ for growing broilers or turkeys has been very limited. Broiler integrators generally feed small particle $CaCO_3$ because of the concern that the abrasive larger particles will cause potential mechanical problems in the feed mill and also affect pellet quality.

The use of feed added phytase is presently being used to help the poultry industry control phosphorus (P) buildup in poultry waste that is used as fertilizer. The phytase enzyme lowers the P in poultry waste and supports an ecological effort to decrease P in our water supply. The amount of Ca in a broiler diet has been reported to affect the use of phytase (Tamim and Angel, 2003). The solubility of phytate P (PP) from feed ingredients in the GI tract has been reported to be affected by pH (Selle *et al*., 2000; Champagne, 1988). Selle *et al*. (2000) have suggested that most PP-mineral complexes are soluble at low pH's (less than 3.5) with maximum insolubility occurring between 4 and 7. Champagne (1988) has reported that Ca-PP complexes precipitate at pH's between 4 and 6 which is the approximate pH of the intestine where the Ca ions should be absorbed. Taylor (1965) has suggested that the primary factor affecting PP utilization is the Ca ion concentration in the small intestine where insoluble Ca-PP-complexes form. A precipitated PP-mineral complex would not be accessible for hydrolysis or absorption in the intestine.

The impact of feeding different particle sizes of $CaCO_3$ on weight gain and feed conversions of broilers has not been studied extensively. Guinote *et al*. (1991) reported that broilers did not show any benefits in weight gain, feed conversions and tibia bone ash when fed coarse particle $CaCO_3$ when comparing several sources. Guinote *et al*. (1991) suggested that the optimum size was finely ground limestone with a particle size less than 150 mm. McNaughton (1981) showed that a medium size particle (USBS 20-60) produced the best weight gain and feed conversions and needed less available P for optimum bone ash compared to smaller particle size $CaCO_3$ (USBS 12 to 20) and larger particle size $CaCO_3$ (USBS 100 to 200).

Table 1: Experimental diets (mash form)

Ingredients	Diet 1 (%)	Diet 2 (%)	Diet 3 (%)	Diet 4 (%)	Diet 5 (%)	Diet 6 (%)	Diet 7 (%)	Diet 8 (%)
Corn, ground, 9.2% CP	61.88	61.88	61.88	61.88	61.88	61.88	61.88	61.88
Soybean meal, 47.5% CP	31.61	31.61	31.61	31.61	31.61	31.61	31.61	31.61
Poultry fat	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Limestone ($CaCO_3$)[2]	1.52	1.52	1.55	1.54	1.55	1.52	1.55	1.52
	(0.03)	(0.03)		(0.01)		(0.03)		(0.03)
Dicalcium phosphate	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
Celite marker[3]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Salt	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49
Choline Chloride (60%)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Lysine HCL	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
DL-Methionine	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21
Threonine	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Vitamin Pre-mix[4]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Trace Minerals[5]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Sacox60[6]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Mold Curb (50% propionic acid)[7]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Ethoxyquin (66%)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Selenium pre-mix (0.06%)[8]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Phyzyme (5000 FTU g^{-1})[9]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

[1]Calculated values of all 8 experimental diets for total P (%), non-phytate P (%), Ca (%), crude protein (%), ME (kcal kg^{-1} diet) are 0.43, 0.20, 0.78, 21.5, and 3025, respectively, [1]Analyzed values of all 8 experimental diets for total P (%), Ca (%), crude protein (%), gross energy (kcal kg^{-1} diet) and dry matter (%) ranged from 0.44-0.46, 0.78-0.96, 19.7-21.8, 3927-4003, and 90.00-90.04, respectively, [2]Values within the parentheses indicate the % of builders sand used as inert material to make up the quantity for limestone inclusion levels since the Ca content of different limestone particle sizes varied from 38.67-39.51%. The average particle sizes of limestone (along with % solubility) used for diets 1 through 8 were 28 (74.4), 137 (56.4), 299 (47.0), 388 (53.0), 519 (46.7), 760 (45.3), 796 (42.2) and 1306 (43.4) µm, respectively. Limestone particles for diets 1, 2, 4, 6, and 8 were sourced from ILC Resources, Alden, IA and for diets 3, 5 and 7 from ILC Resources, Weeping Water, NE, [3]Celite Corp., Lompoc, CA 93436, [4]Vitamin mix provided per kg of diet: vitamin A (vitamin acetate), 7709 IU; Vitamin D3, 3304 ICU; vitamin E, 16.5 IU; niacin, 38.6 mg; D-pantothenic acid, 9.9 mg; riboflavin, 6.6 mg; pyridoxine (pyridoxine HCl), 2.75 mg; thiamine (thiamine mononitrate), 1.54 mg; menadione (menadione nicotinamide bisulfite), 1.5 mg; folic acid, 0.88 mg; biotin, 0.066 mg; vitamin B12, 0.013 mg; ethoxyquin, 132 mg; selenium, 0.1 mg, [5]Trace mineral mix provided per kg of diet: manganese ($MnSO_4.H_2O$), 100mg; zinc ($ZnSO_4.7H_2O$), 100 mg; iron ($FeSO_4.7H_2O$), 50 mg; copper $CuSO_4.5H_2O$), 10 mg; iodine ($Ca(IO_3)_2.H_2O$, 1 mg; magnesium (magnesium oxide), 26.5 mg, [6]Intervet, Inc., Millsboro, DE 19966, [7]Kemin Industries, Inc., Des Moines, IA 50317, [8]Prince Agric Products, Inc., One Prince Plaza-Quincy, IL 62305, [9]Danisco Animal Nutrition, Marlborough, Wiltshire, UK. The analyzed activity of phytase in Phyzyme XP preparation was 5260 FTU g^{-1} that provides 526 FTU kg^{-1} diet compared to the expected value of 500 when added to the diet @0.01%

The solubility of Ca in the gastrointestinal tract may have a direct affect on forming PP-mineral complexes. Research has neglected to evaluate the effects of Ca particle size and solubility on PP hydrolysis with an exogenous phytase in broilers. Broilers may gain more from feeding phytase by feeding larger particle $CaCO_3$ with lower solubility to minimize the solubility of the $CaCO_3$ in the crop and in anterior portion of the gastrointestinal tract. A low solubility form of $CaCO_3$ may allow the phytase enzyme more accessibility to the PP in the gut and provide more available P from PP hydrolysis in the broiler.

The objectives of the present research were: a) to evaluate the impact of feeding different particle sizes of $CaCO_3$ on broiler performance and tibia ash and b) to determine the effect of different particle sizes of $CaCO_3$ on PP hydrolysis *in vitro* at pHs of 2.5 and 6.5.

Materials and Methods

Sixteen hundred and eighty Cobb 500[2] male day old chicks were assorted into 48 floor pens with 35 chicks per pen. The broilers were fed a corn soybean meal based diets (Table 1) containing approximately 21.5% CP, 3025 kcal ME kg^{-1}, 0.78% Ca, 0.20% NPP with 500 FTU/kg of Danisco Phyzyme XP[3] added. The experimental diets were not pelleted to eliminate problems with enzyme destruction or problems associated with pelleting from the large particle treatments. The broilers were divided into 8 treatments with 6 replicate floor pens per treatment and fed the experimental diets for a 28-day period. Chicks were provided 24 hr access to both water and feed during the entire experimental period. After first two days of 24 h light, a 23:1 h light to dark schedule was provided. The eight dietary treatments were based on feeding 8 different particle sizes of limestone ($CaCO_3$) with different solubility % (Table 1). The average particle sizes of $CaCO_3$ (along with % solubility) tested in diets 1 through 8 were 28 (74.4), 137 (56.4), 299 (47.0), 388 (53.0), 519 (46.7), 760 (45.3), 796 (42.2) and 1306 (43.4) µm, respectively. The only difference across the diets was change in the addition of different particle size $CaCO_3$. The $CaCO_3$ added for diets-1, 2, 4, 6 and 8 were procured from the ILC Resources, Alden[4] and for diets 3, 5 and 7 were procured from the ILC Resources, Weeping Water[5] (Table 1). The NPP was set at 0.20% to maximize the response differences between the different Ca sources. The suggested NRC (1994) requirement for NPP for broiler chicks from hatching to 3 wk of age is 0.45%. If the test diets contain too much NPP then it would be more difficult to detect a significant difference in broiler performance and bone development. In order to produce a maximum difference in the treatments, the broilers were fed 0.20% NPP levels that would produce a linear growth and bone ash response with additional available P coming from the hydrolyzed PP. The Phyzyme XP (@ 500 FTU kg^{-1} diet) used in the study should provide 0.13% available P or the equivalent NPP based on the study of Coon and Manangi (2004). An optimum Ca source because of particle size and solubility would minimize the amount of PP that precipitates or forms a mineral PP complex. The more PP that stays in solution in the GI tract, the more opportunity phytase will have for PP hydrolysis creating available Ca and P for growth and bone ash formation. The phytase response should provide an additional 0.13% available P. The Ca level was 0.90% which includes 0.78% from feed ingredients and 0.11% Ca from phytase hydrolysis of the phytate complex based on the study of Coon and Manangi (2004).

The broilers were fed the experimental diets for 28 days and weighed at termination. One percent Celite (Table 1) was used as a digestibility marker in all the experimental diets. Feed consumptions were determined for each replicate and feed:gain (F:G) were determined. The mortality for each replicate was determined and the F:G adjusted. On d-28, 288 chicks (6 chicks from each pen) were selected randomly from the floor pens and euthanized by CO_2 inhalation. Digesta samples were collected from the ileum from each bird, pooled for each replicate pen, freeze-dried and ground for analysis. The ileum was defined as that portion of the small intestine extending from the Meckel's diverticulum to a point approximately 40 mm to the ileo-cecal junction. Tibiae (right and left) were taken from each bird, cleaned of all exterior tissue and frozen for later analysis. The tibiae from six broiler chicks representing each floor pen were pooled for ash analysis. Animal use protocol for the present experiment was approved by the University of Arkansas Institutional Animal Care and Use Committee (IACUC).

Diets and ileal digesta samples were analyzed for total P and Ca by inductively coupled plasma emission spectroscopic method as mentioned by Leske and Coon (2002). Acid insoluble ash was determined in experimental diets and ileal digesta samples using dry ash and hydrochloric acid digestion technique of Scott and Balnave (1991). The feed and ileal digesta samples were analyzed for moisture by standard AOAC procedures (1990). Retention and or digestibility values of dietary total P (TP) and Ca were determined by using the acid insoluble ash concentrations with a marker digestibility equation reported by Scott and Balnave (1991). Ash per tibia (mg) and tibia ash % were estimated on defatted dried tibiae. Phyzyme XP preparation was assayed for phytase from Danisco Animal Nutrition, Marlborough, United Kingdom. The % solubility for different $CaCO_3$ particle sizes was determined at ILC Resources using Zhang and Coon (1997) method.

For the *in vitro* study, the general procedures of Tamim and Angel (2003) were followed with modifications that include use of different $CaCO_3$ particles and doubling the quantity of incubation mixture. In brief, the effect of Ca particle sizes on PP hydrolysis by a 3-phytase enzyme from *Aspergillus ficuum*[6] with a pH optimum of 5 was determined for five different intervals at 15, 30, 60 and 120 min periods at pHs of 2.5 and 6.5. The level of Ca tested was equivalent (assuming feed to water consumption ratio of 1:2) to 0.9 g kg^{-1} diet, the level used for the present broiler performance study. A 4.62 g/L sodium phytate[6] solution (929 mg phytate P/L) in 200 mM glycine buffer (pH 2.5) or 200 mM acetate buffer (pH 6.5) was used as the substrate. Phytase enzyme solution was prepared by suspending an appropriate quantity of *Aspergillus ficuum* enzyme product in glycine and acetate buffer such that 100-μL volume would contain the equivalent of 500 U of phytase kg^{-1} diet when added to the substrate solutions. A 200-μL volume of phytase enzyme solution was added to a test tube containing 6 mL of the substrate solution and $CaCO_3$ (0.0464 g) particles. The resulting mixtures were incubated at 37°C for 0, 15, 30, 60 and 120 min. All incubations were in triplicates and each one of these triplicate incubations served as the experimental unit. At the end of each incubation period, the reaction was stopped by the addition of 4 mL of ammonium molybdate-metavandate reagent (Chen, 1996) and liberated P was measured spectrophotometrically at 410 nm according to Heinonen and Lahti (Heinonen and Lahti, 1981). The inorganic P[6] was used as a standard.

Statistical analysis: Data from the broiler experiment were subjected to 1-way analysis of variance (ANOVA) to determine overall effect of feeding different particle sizes of $CaCO_3$ and the data from the *in vitro* study were subjected to 2-way ANOVA to determine the main effects of phytate and phytase and their interaction using the GLM procedure (SAS, 1999). In both the experiments means were compared using least significant difference tests.

Results and Discussion

Body Weight Gain (BWG) and feed consumption were significantly ($p<0.0001$) affected by feeding different particle size $CaCO_3$ (Table 2). F:G was not significantly

Table 2: The effect of feeding different particle sizes of limestone with added phytase on the performance, mortality, ileal nutrient digestibility and bone ash in chicks during 28-d feeding trial[1]

Limestone	BWG kg/chick	FI kg/chick	F:G	Chick Mortality%	Total P intake mg	Total P retention mg	Total P retention%	Ca retention%	Bone Ash%	Ash/tibia mg
Particle sizes (μm)										
Diet-1 (28)	1.01^{c}	1.47^{bc}	1.59	0.00^{c}	6677^{bc}	3393^{bc}	51.06	69.17^{ab}	44.39	992^{b}
Diet-2 (137)	1.12^{a}	1.67^{a}	1.54	0.00^{c}	7576^{a}	4271^{a}	56.4	66.47^{abc}	44.85	1030^{ab}
Diet-1 (299)	1.12^{a}	1.71^{a}	1.57	1.45^{bc}	7763^{a}	3853^{ab}	49.63	62.47^{bc}	45.60	1050^{ab}
Diet-7 (388)	1.11^{ab}	1.59^{ab}	1.51	2.40^{b}	7210^{ab}	3529^{bc}	52.87	62.18^{bc}	45.36	1096^{a}
Diet-2 (519)	0.95^{cd}	1.40^{cd}	1.59	0.00^{c}	6384^{cd}	3202^{c}	50.61	64.11^{abc}	45.38	1031^{ab}
Diet-6 (760)	1.03^{bc}	1.49^{bc}	1.54	0.48^{bc}	6788^{cb}	3500^{bc}	54.28	61.53^{c}	44.40	999^{ab}
Diet-3 (796)	0.97^{cd}	1.40^{cd}	1.59	1.91^{bc}	6354^{cd}	3271^{bc}	51.36	61.29^{c}	44.95	962^{b}
Diet-8 (1306)	0.89^{d}	1.27^{d}	1.63	9.52^{a}	5790^{d}	3302^{bc}	56.84	70.49^{a}	44.13	950^{b}
Pooled SEM	0.03	0.05	0.03	1.75	212	221	2.6	2.58	0.64	35.42
p value	<0.0001	<0.0001	0.1663	<0.0001	<0.0001	0.0246	0.3646	0.0842	0.6539	0.1045

[1]Means within columns with no common superscripts differ significantly ($p<0.05$), [1]For BWG (Body Weight Gain), FI (Feed Intake) and F:G (feed to gain ratio), means are an average of 6 replicate pens per treatment with 35 chicks/pen and for all other parameters except mortality, means are an average of 6 replicate pens per treatment with 6 chicks/pen

($p = 0.1663$) affected by $CaCO_3$ particle size. The best performance in terms of BWG and F:G was obtained for chicks fed $CaCO_3$ particle size of 388 μm. A comparable BWG response was also obtained for chicks fed $CaCO_3$ particle size of 137 and 299 μm. The chicks fed the largest $CaCO_3$ particle size of 1306 μm had gained least weight (0.89 kg) with the poorest F:G (1.63) and the highest mortality percentage (Table 2). TP retention (%) was not significantly ($p = 0.3646$) affected by $CaCO_3$ particle size but the total quantity of P intake ($p = 0.0017$) and the total quantity of P retained ($p<0.0001$) were significantly affected (Table 2). The increase in the performance (BWG) could be attributed to increased quantity of P consumed and retained in chick groups fed average $CaCO_3$ particle sizes of 137 , 299 and 388 μm. The results (Table 2) show that $CaCO_3$ particle size has a tendency to affect' mg of ash/tibia' ($p = 0.1045$) and retention (%) of Ca ($p = 0.0842$), but not percent bone ash ($p = 0.6539$). The total' mg of ash/tibia' was highest for the group of chicks fed $CaCO_3$ particle size of 388 μm. The report of Guinote *et al.* (1991) indicates that ground particles of Ca (less than 0.15 mm) significantly improved performance and ossification characteristics of tibiae in broiler chicks during 28 d feeding trial. The Guinote *et al.* (1991) study had three different particle sizes categorized as ground (less than 0.15 mm), medium (0.6 to 1.18 mm) and coarse (greater than 1.18 mm). Our findings from the present study indicate the average Ca particle size that ranges from 137 to 388 μm utilized in the experimental diets with phytase supplementation improved chicks performance for a 28 day feeding study. McNaughton (1981) reported greater tibia ash and body weight for 21-d old chicks fed corn-soybean meal diets containing medium size Ca particles (USBS 20-60, 250-840 μm) than when either the USBS 12-20 (840-1700 μm) or 100-200 (70-150 μm) particle-sizes of $CaCO_3$ were fed. The present study cannot be compared with previous literature reports for determining the optimum Ca particle size for all situations because the earlier research consisted of using different Ca sources and particle sizes with unknown solubility and the previous work did not include the addition of phytase to the test diets. Previous literature reports by Guinote *et al.* (1991) and McNaughton (1981) indicate Ca utilization by the chick is dependent upon the particle size of the Ca supplement and the most desirable particle sizes of $CaCO_3$ ranged from a fine to medium particle size for producing optimum chick weight and percent tibia ash.

The results of the *in vitro* study carried out to evaluate the effect of different Ca particle sizes and pH on PP hydrolysis at 15, 30, 60 and 120 min incubation at 37°C are given in Table 3. Data shows significant effect of particle size ($p<0.05$), pH ($p<0.05$) and interaction ($p<0.05$) of particle size and pH on PP hydrolysis by phytase in releasing inorganic P at all four incubation periods. The exception was the main effect of pH ($p = 0.5271$) at 120 min.

The range of values for PP hydrolysis (μg P released/unit phytase) is slightly higher for incubations from 15 min through 120 min at both pHs (Table 3) compared to the *in vitro* study of Tamim and Angel (2003) that utilized one particular size $CaCO_3$. The differences in the quantity of inorganic P released in the present *in vitro* study as compared to the *in vitro* study of Tamim and Angel (2003) at the tested level of equivalent to 9 g kg^{-1} feed could be due to the differences in the particle size of $CaCO_3$ used, addition of Ca to PP solution in the incubation mixture and the duration of mixing and stirring. The main effect of pH shows the PP hydrolysis at 15 and 30 min incubation is reduced by 33% and 15%, respectively, at pH 6.5 compared to PP hydrolysis at pH 2.5. The negative influence of mineral cations, including Ca, on PP hydrolysis at a higher pH has been reported in the literature (Tamim and Angel, 2003; Grynspan and Cheryan, 1983; Gifford and Clydesdale, 1990; Maenz *et al.*, 1999). The PP hydrolysis was reduced 8% in the *in vitro* assay when incubation mixture was pH 2.5 and contained the smallest particle size $CaCO_3$ compared to PP hydrolysis from mixture with

Table 3: Effect of Ca particle size and pH on *in vitro* phytate P hydrolysis[1]

Limestone particle sizes (μm)	pH	μg P released/unit phytase 15 min	30 min	60 min	120 min
28	2.5	567^{d}	613^{b}	735^{cd}	726de
137	2.5	622^{b}	657^{a}	766^{a}	783^{a}
299	2.5	609^{bc}	665^{a}	727^{cd}	723de
388	2.5	604^{bc}	668^{a}	669^{g}	791^{a}
519	2.5	656^{a}	653^{a}	763^{ab}	727de
760	2.5	601^{c}	654^{a}	700^{ef}	773^{ab}
796	2.5	606^{bc}	656^{a}	732^{cd}	713^{e}
1306	2.5	607^{bc}	667^{a}	714de	777^{ab}
28	6.5	367^{g}	506^{d}	644^{hi}	735^{cde}
137	6.5	400^{f}	603^{b}	645^{hi}	736^{cd}
299	6.5	368^{g}	516^{d}	667^{gh}	755^{bc}
388	6.5	418^{f}	552^{c}	742^{bc}	732de
519	6.5	399^{f}	567^{c}	636^{i}	776^{ab}
760	6.5	404^{f}	542^{c}	640^{i}	777^{ab}
796	6.5	444^{e}	550^{c}	684^{gf}	739^{cd}
1306	6.5	458^{e}	594^{b}	682^{gf}	784^{a}
Pooled SEM		7	9	8	8
Particle size effect					
28		467^{e}	560^{d}	690^{b}	730^{e}
137		511^{bc}	630^{a}	706^{ab}	760^{bc}
299		488^{d}	591^{c}	697^{ab}	739de
388		511^{bc}	610^{b}	706^{ab}	762^{bc}
519		527^{a}	610^{b}	699^{ab}	752^{cd}
760		503^{c}	598^{bc}	670^{c}	775^{ab}
796		525^{ab}	603^{bc}	708^{a}	726^{e}
1306		533^{a}	631^{a}	698^{ab}	780^{a}
pH effect					
2.5		609	654	726	752
6.5		407	554	668	754
Source of variation					
Particle size effect		<0.0001	<0.0001	0.0006	<0.0001
pH effect		<0.0001	<0.0001	<0.0001	0.5271
Particle size x pH		<0.0001	0.0003	<0.0001	<0.0001

[1]Means within columns with no common superscripts differ significantly ($p<0.05$), [1]Means are an average of 3 replicates per incubation period

the largest particle size of $CaCO_3$ (Table 3). The PP hydrolysis was reduced 15% when incubation mixture was pH 6.5 and contained the smallest particle size $CaCO_3$ compared to PP hydrolysis from mixture with the largest particle size of $CaCO_3$ (Table 3).

A low solubility form of $CaCO_3$ may allow the phytase enzyme more accessibility to the PP in the gut and provide a higher apparent PP hydrolysis for the broiler. The improved performance of chicks fed $CaCO_3$ with an average particle size ranging between 137 to 388 μm could be due to maximum response of phytase. The $CaCO_3$ with very small particles has a high solubility and may pass through the gastrointestinal tract at a faster rate and decrease maximum retention. The highly soluble Ca from the small particles may also enhance the formation of a mineral-PP complex that limits the ability of added dietary phytase to hydrolyze PP. Guinote *et al.* (1991) showed that feeding broilers large particle $CaCO_3$ (1.18 to 4.75 mm) decreased Ca retention by 6 to 7 percentage points compared to feeding $CaCO_3$ with particle sizes either 0.3 to 1.18 mm or less than 0.15 mm. The researchers found no difference ($p>0.05$) in Ca retention in birds fed either small (less than 0.15 mm) or medium (0.3 to 1.15 mm) size $CaCO_3$ particles. The present feeding study utilized 8 different $CaCO_3$ particle sizes ranging from 28 to 1306 μm and these sizes were comparable to the small and medium size Ca particles utilized in the Guinote *et al.* (1991) study. The present research shows an overall trend ($p = 0.0842$) that particle size affected broiler Ca retention, however in contrast to the findings of Guinote *et al.* (1991) the Ca retention was numerically the highest for chicks fed the test diet containing $CaCO_3$ with the largest average particle size of 1306 μm. Guinote *et al.* (1991) suggested the calcium retention was lower for broilers fed larger $CaCO_3$ particles because the particles were retained in the gizzard. Rao and Roland (1989) previously have showed that it takes a particle size of 900 μm to be large enough to be retained by the gizzard of a laying hen. Larger particles produce higher calcium retention in laying hens compared to smaller particles because of increased utilization of dietary calcium for shell formation; however, there is little data comparing the Ca retention of different particle sizes for growing broilers. The overall performance of the chicks fed the large particle $CaCO_3$ in the present research was less than chicks fed smaller particles of $CaCO_3$ because of less feed intake. The larger particle retained in the gizzard may not have provided adequate calcium intake for optimum performance and tibia ash. The broilers fed the largest $CaCO_3$ particle size in the present feeding study had the highest % Ca retention and also the highest % total P retention. The broilers fed the largest Ca particles are probably compensating for the poor intake of nutrients with increased retention and nutrients absorption at low levels of feed intake. Another possible reason for improved retention of Ca and P for broilers fed the largest $CaCO_3$ particles could be due to an improved benefit from dietary phytase. The slow release of Ca from the larger Ca particle size due to less solubility may have reduced the formation of Ca-phytate complex and improved phytate hydrolysis in the lower part of the gut. Guinote *et al.* (1991) suggested the longer transit time needed for course particles of $CaCO_3$ to pass through the gastrointestinal tract of broilers because of gizzard retention and the lower solubility of the particles might explain the negative effect of large or coarse particle size (1.18 to 4.75 mm) $CaCO_3$. In conclusion, the results of the present study shows that a $CaCO_3$ particle size between 137 and 388 μm fed in broiler diets with added phytase (500 FTU kg^{-1} diet) will produce the best BWG, F:G, and 'mg of ash/tibia' in broilers during the first 28 days. Both *in vivo* and *in vitro* phytase studies indicate that a small Ca particle size (28 μm) $CaCO_3$ with a high solubility (>70.0%) limits PP hydrolysis for releasing available P for growth and bone ash formation.

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