Assessment of postcrumble addition of limestone and calcium-specific appetite in broilers during the starter phase[1]

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ABSTRACT A study was done to determine whether broilers can regulate Ca intake when limestone is provided separately or mixed with a crumbled feed of variable Ca and P content, and the influence of this on performance and apparent ileal digestibility (AID) of Ca and P (AIDP). Twelve crumbled diets were fed from 10 to 20 d of age (8 replicates, 8 broilers/replicate). Diets A to D contained 0.28% nonphytate P (nPP) and 0.27, 0.51, 0.77, and 1.02% Ca, respectively. Diets E to H contained 0.48% nPP and 0.41, 0.51, 0.77, and 1.02% Ca, respectively. A large particle size limestone was mixed manually to the crumbled diet on a daily basis to achieve 1.02% total Ca in diets A to H. Diets I to L had the same Ca and nPP as diets A to D, but limestone was provided in a separate feeder to assess spatial importance of limestone supply. Limestone consumption, provided in a separate feeder, decreased as Ca concentration increased in the crumble diet ($P < 0.05$). Calcium intake increased as Ca concentration in crumbled diets increased ($P < 0.05$). Increased tibia ash and decreased AIDP were observed as Ca intake increased ($P < 0.05$). When limestone was added to diets containing 0.28% nPP postcrumble, Ca intake (6.38 g/bird), tibia ash (717 mg/bone), and AIDP (39.78%) were not affected by crumbled diet Ca concentration or consumed Ca. Broilers fed diets containing 0.48% nPP and limestone mixed with the crumble, Ca intake changed (5.96, 6.93, 6.59, and 6.04 g/bird for crumble diet with 0.41, 0.51, 0.77, and 1.02% Ca, respectively). Increasing Ca concentration in the crumble from 0.41 to 1.02% resulted in greater tibia ash (875 mg/bone) but lower AIDP ($P < 0.05$), although Ca intake was similar. In conclusion, when large particle size limestone was provided ad libitum, the ability of broilers to select for Ca was not sufficient to meet their requirement when crumble Ca was less than 0.77%. The AIDP was highest in birds fed the 0.27% Ca concentration diet.

Key words: broiler, digestibility, limestone, separate feeding, bone ash

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INTRODUCTION

Phosphorus makes up approximately 30% of skeleton ash content (Angel, 2007; Shastak, 2012) and is also involved in a variety of metabolic pathways. Dietary Ca concentration has a strong impact on the availability of P in broiler chickens. A study by Wilkinson et al. (2014) reported that apparent ileal digestible P increased from 0.27 to 0.37% when dietary Ca was reduced from 1.0 to 0.25%. Similar results were reported by Tamim and Angel (2003) and Tamim et al. (2004) where when the dietary Ca was reduced from 0.52 to 0.12% (Tamim and Angel, 2003) or 0.65 to 0.18% (Tamim et al., 2004), disappearance of phytate-P up to the distal ileum increased from 18.1 to 67.1% (Tamim and Angel, 2003) or 25.4 to 69.2% (Tamim et al., 2004), showing that reducing dietary Ca concentration results in an increase in the digestibility P by improving phytate-P digestibility, presumably via increased persistency of phytate solubility in the small intestine.

Work by Wilkinson et al. (2014) confirmed previous reports (Wood-Gush and Kare, 1966; Hughes and Wood-Gush, 1971; Joshua and Mueller, 1979) that broilers have a specific appetite for Ca. Their results showed that when broiler chickens were fed diets containing 0.25 to 1.0% Ca and additional large particle size (diameter = 2 mm) limestone was provided in a separate feeder, broilers chose to consume more limestone to obtain a similar Ca concentration regardless of

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the Ca in the feed. This conclusion corroborates previous findings where Ca appetite was reported in poultry (Wood-Gush and Kare, 1966; Hughes and Wood-Gush, 1971; Joshua and Mueller, 1979). The difficult logistics and low acceptance by industry of feeding limestone in separate feeders prompted the work presented in the current paper as a follow-up to Wilkinson et al. (2014). Postcrumble limestone addition, an approach that has some precedent in the postcrumble addition of whole wheat, could be a strategy that allows for similar results as those reported by Tamim et al. (2003) and Wilkinson et al. (2014). The objectives of the current study were therefore to determine 1) whether broilers can maintain a Ca intake target when limestone is provided for ad libitum consumption in a separate feeder or mixed with a crumbled feed of variable Ca and P content, 2) the effect of Ca choice by the broilers on apparent ileal digestibility (**AID**) of Ca and P (**AIDCa** and **AIDP**), and 3) the effects of dietary nonphytate (**nPP**) concentration on total Ca intake and the ability of the broilers to choose Ca.

MATERIALS AND METHODS

Birds and Housing

All animal care procedures were approved by the University of Maryland Animal Care and Use Committee. A total of 768 straight run Hubbard 99 M × Cobb 500 F broiler chicks were obtained from a local commercial hatchery (Allen Harim Farms, Seaford, DE) and placed in an artificially lit and environmentally controlled room upon arrival. Birds were fed a commercial type starter diet that met or exceeded all NRC (1994) recommendations as well as average nutrient usage concentrations in the United States for 2011 (AgriStats, 2012, end of year summary). At 10 d of age, birds were weighed individually, and groups (pens) of 8 birds were put together such that both pen to pen and within pen chick weight variation were minimized. The groups were placed randomly into battery pens (Petersime Incubator Co., Gettysburg, OH) previously assigned to treatment (**Trt**). Treatments were assigned such that each of the 4 rooms was a block and each treatment was replicated at least twice in each room. The wire-floored battery pens (width × depth × height; 99.7 cm × 68.6 cm × 29.2 cm), were equipped with a water nipple system (2 nipples per pen) and 2 feed troughs (width × depth × height; 63.5 cm × 8.9 cm × 5.67 cm). The light program was 24L:0D from hatch to 3 d, 14L:10D from 4 to 10 d, and 18L:6D from 11 to 20 d of age. Room temperature was kept at an average of 31°C from hatch to 3 d and brooder lamps were used to provide additional heat. Room temperature was lowered to 29°C from 4 to 8 d, 27°C from 9 to 14 d, and 25.5°C from 14 to 20 d of age. Mortalities were checked twice a day, bird weight as well as feed and the rest of the birds in the pen weighed and weights used to correct BW gain (**BWG**), feed intake (**FI**), and feed conversion ratio (**FCR**). Chicks had free access to feed and water throughout the trial.

Experimental Design and Diets

The trial was a randomized block design with 12 Trt, 8 replicates per Trt, and 8 birds per replicate. A corn and soybean meal mashed basal diet that contained 10% wheat, which was included to improve the pellet quality, was mixed (Table 1) and analyzed for DM, minerals, protein, ether extract, and amino acids. Based on the analyzed Ca and P concentrations in the basal diet, preanalyzed limestone [Limestone #20 (IMI Cal Pro, Greenfield, IN); mean diameter ($\mathbf{d_{gw}}$) = 0.402 mm; geometric SD ($\mathbf{S_{gw}}$) = 0.255 mm] and monocalcium phosphate [$Ca(H_2PO_4)_2$ (Monocal, Kirby Agri, Mechanicsville, MD); d_{gw} = 0.759 mm; S_{gw} = 0.258 mm] were added to achieve the desired Ca and nPP concentrations in the final diets. The basal diet was included in all final diets at 96.7% of the total. Chromic oxide was added as an indigestible marker at 0.4% and Celite (World Minerals, Santa Barbara, CA) was included as a filler to achieve 100%. Ingredient and nutrient composition of the basal diet are presented in Table 1. Treatments A (0.29% Ca; 0.28% nPP), B (0.51% Ca; 0.28% nPP), C (0.77% Ca; 0.28% nPP), D (1.02% Ca; 0.28% nPP), E (0.41% Ca; 0.48% nPP), F (0.51% Ca; 0.48% nPP), G (0.77% Ca; 0.48% nPP), and H (1.02% Ca; 0.48% nPP) were formulated, mixed, pelleted, and then crumbled. Conditioner temperature was kept at 71°C for all batches. The average particle size (mm) of the crumbled diets was 1.969 mm (S_{gw} = 1.088 mm). Feed was weighed into feeders for all treatments every 1 or 2 d. For Trt A to H, a separate source of large particle size limestone [Limestone #12 (IMI Cal Pro); d_{gw} = 1.988 mm; S_{gw} = 1.651 mm] was weighed to achieve the total Ca desired in the diet (1.02%) and manually mixed with the crumbled diets to minimize changes in particle size. For diets I to L, the same crumbled diets as for diets A to D were used, but instead of mixing large particle size limestone with the crumble it was offered in a separate feeder for free choice consumption.

Performance

Pen BW was determined at 10 and 20 d of age. Feed disappearance from feeders for each pen was recorded on d 12, 14, 17, 19, and 20. Because it was not possible to separate the large particle size limestone from the crumbled feed when it was mixed with the diets (Trt A–H), feed and large particle size limestone wastage found in the excreta pan were collected together into the same container. When large particle size limestone was provided in a separate feeder, feed and limestone wastage were collected in 2 separate containers. Wastage was weighed daily, pooled by pen through the whole experiment, and analyzed for DM, Ca, and P. These values were used to determine true Ca intake by subtracting the wastage Ca amounts from the amounts

Table 1. Ingredient and chemical composition of the basal diet

Item	Basal, % (as-fed basis)
Ingredient	
Corn	48.32
Soybean meal (48% CP)	36.37
Wheat	10.00
Soy oil	3.18
Salt	0.52
Monocalcium phosphate[1]	0.43
Limestone #20[2]	0.04
DL-Methionine, 99%	0.36
Biolys, 55%	0.37
L-Threonine, 98.5%	0.08
Choline chloride, 60%	0.16
Mineral premix[3]	0.08
Vitamin premix[4]	0.08
Total	100.00
Formulated (analyzed) concentrations, % (except where specified)	
ME_n, kcal/kg	3,102
CP	22.72 (23.49)
Lysine	1.43 (1.40)
Methionine + cysteine	1.06 (1.05)
Ca	0.22 (0.24)
Total P (tP)	0.52 (0.56)
Phytate P (PP)	0.30 (0.32)
Nonphytate P[5] (nPP)	0.22 (0.24)

[1]Monocal, Kirby Agri, Mechanicsville, MD. Analyzed Ca and P: 16.06 and 21.95%, respectively. Mean diameter (d_{gw}) = 0.759 mm; geometric SD (S_{gw}) = 0.258 mm. Distribution: <0.075 mm, 0.34%; 0.075 to 0.250 mm, 0.90%; 0.250 to 0.300 mm, 0.27%; 0.300 to 0.500 mm, 3.81%; 0.500 to 0.600 mm, 9.13%; 0.600 to 0.710 mm, 17.22%; 0.710 to 0.850 mm, 24.04%; 0.850 to 1.000 mm, 34.83%; 1.000 to 1.180 mm; 8.64%; >1.180 mm, 0.82%.

[2]Small particle size limestone (IMI Cal Pro, Greenfield, IN). Analyzed Ca, 36.65%. Particle size, mean diameter (d_{gw}) = 0.402 mm; geometric SD (S_{gw}) = 0.255 mm. Distribution: <0.075 mm; 1.72%; 0.075 to 0.150 mm, 4.99%; 0.150 to 0.250 mm, 10.20%; 0.250 to 0.300 mm, 7.88%; 0.300 to 0.355 mm, 9.36%; 0.355 to 0.425 mm, 13.10%; 0.425 to 0.500 mm, 10.84%; 0.500 to 0.600 mm, 15.96%; 0.600 to 0.710 mm, 9.74%; 0.710 to 0.850 mm, 5.36%; 0.850 to 1.000 mm, 5.36%; >1.000 mm, 0.77%.

[3]Supplied per kilogram of diet: zinc from zinc sulfate, 80 mg; manganese from manganese sulfate, 100 mg; iron from iron sulfate, 20 mg; copper from copper sulfate, 3 mg; iodine from calcium iodate, 3.9 mg; selenium from selenium sulfate, 0.3 mg.

[4]Supplied per kilogram of diet: vitamin A, 15,111 IU; vitamin D, 5,333 IU; vitamin E, 53.33 IU; vitamin B_{12}, 26.66 mg; riboflavin, 17.78 mg; niacin, 71.11 mg; pantothenic acid, 24.89 mg; vitamin K_3, 3.2 mg; folic acid, 2.13 mg; biotin, 0.142 mg; thiamine, 4.44 mg; pyridoxine, 6.22 mg.

[5]Determined nPP based on analyzed tP minus analyzed PP.

of Ca from the feed or large particle size limestone that disappeared. Feed intake, BW, BWG, and FCR for the whole experimental period were adjusted for mortality and calculated on a per bird basis. The pen of 8 birds was the experimental unit.

Sample Collection

All birds in the pen were euthanized by cervical dislocation at 20 d of age. The last half of the ileum (distal half of the small intestinal segment encompassed between Meckel's diverticulum to ileocecal junction) was removed and the content gently expressed by flushing with distilled water. The contents were pooled by pen, frozen at −20°C, and freeze-dried. Dried ileal digesta samples were ground using a mortar and pestle to pass through a 0.5-mm sieve and stored in airtight containers at 4°C until analyzed. Middle toes from both feet of each bird were cut off at the third metatarsal and right tibias removed from all birds in the pen. Tibias were cleaned of flesh and cartilaginous caps removed. Toes and tibias were oven-dried at 100°C for 24 h, defatted by refluxing petroleum ether in a Soxhlet apparatus for 16 h, oven-dried at 100°C overnight for dry defatted bone weight determination, and ashed in ceramic crucibles for 16 h at 600°C (method 972.15; AOAC, 1990). Ash content was determined on a dry, fat-free basis, and expressed either as milligrams per bone or percentage of the dry defatted bone weight.

Laboratory Analysis

Diets were ground to pass through a 1-mm screen before analysis. Dry matter of the diets were determined by drying overnight in a 100°C oven (Shreve et al., 2006). Diet, leftover feed, wastage, and ileal Ca and P were determined after acid digestion and analyzed using inductively coupled plasma atomic emission spectrometry (AOAC International, 1999). Amino acid concentrations in the basal diet were determined by performic acid oxidation with the acid hydrolysis sodium metabisulfite method (method 994.12, AOAC International, 1997). Phytate-P in the basal diet was analyzed based on the method of Vinjamoori et al. (2004). Chromic oxide concentration in diets and ileal content were determined by the method described by Fenton and Fenton (1976). Particle size distribution for ingredients and all crumble diets were determined as described by ASABE (2006) method S319.3.

Calculations

The AID was calculated based on the following formula with Cr_2O_3 being used as the indigestible marker:

$$\text{apparent ileal digestibility} = \frac{(\text{Min}/\text{Cr}_2\text{O}_3)_\text{d} - (\text{Min}/\text{Cr}_2\text{O}_3)_i}{(\text{Min}/\text{Cr}_2\text{O}_3)_\text{d}} \times 100\%,$$

where $(\text{Min}/\text{Cr}_2\text{O}_3)_\text{d}$ is the ratio of minerals (Ca or P) to Cr_2O_3 in the diet and $(\text{Min}/\text{Cr}_2\text{O}_3)_\text{i}$ is the ratio of minerals (Ca or P) to Cr_2O_3 in the ileal digesta.

Total Ca intake per bird when large particle size limestone was added to the diets postcrumble was calculated as follows:

$$\text{total Ca intake}(\text{g/bird}) = \frac{[\text{Wt}_\text{diet} \times \%\text{Ca}_\text{diet} - (\text{Wt}_\text{leftover} + \text{Wt}_\text{wast}) \times \%\text{Ca}_\text{lw}]}{\text{n}},$$

where $\%Ca_{diet}$ is the overall Ca concentration in each diet, which is the sum of analyzed Ca in crumble and Ca in large particle size limestone. Diets A through H should all contain a total of 0.92% Ca, but where the Ca is coming from varies between these diets. The Wt_{diet} is total feed disappearance (total crumbled feed + large particle size limestone), $Wt_{leftover}$ is the weight of feed that was left in the feeder, Wt_{wast} is the weight of feed limestone that was collected from the excreta pan, $\%Ca_{lw}$ is the Ca concentration in the pooled leftover and wastage feed, and n is the number of birds in each pen.

The total Ca intake per bird when large particle size limestone was provided separately (Trt I–L) was calculated using the following formula:

$$\text{total Ca intake}(\text{g/bird}) = \frac{[Wt_{\text{crumble intake}} \times \%Ca_{\text{crumble}} + Wt_{\text{limestone intake}} \times \%Ca_{\text{limestone}}]}{n},$$

where the $Wt_{\text{crumble intake}}$ is the total crumbled feed that disappeared from the feeder minus wastage crumble feed, $\%Ca_{\text{crumble}}$ is the Ca concentration in crumbled feed, $Wt_{\text{limestone intake}}$ is the weight of limestone disappearance minus weight of the limestone wastage, $\%\ Ca_{\text{limestone}}$ is the Ca concentration in large particle size limestone, and n is the number of birds in each pen.

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., 2008). Treatments were considered as a fixed effect and pen as a random effect. In addition, a 3 × 3 factorial analysis with 3 Ca supply strategies and 3 Ca concentrations was also performed on selected treatments to determine the main effects and their interaction. The Ca supply strategies in this trial were categorized as 1) postcrumble, low nPP (Trt B–D); 2) postcrumble, high nPP (Trt F–H); and 3) separate feeder, low nPP (Trt J–L). Diets with the lowest formulated Ca concentration from each Ca supply strategy (Trt A, E, and I) were excluded from the factorial analysis because one of the basal diets needed to make diet E was analyzed higher than expected and thus diet E could not be made to the low Ca concentration. In the experimental design, diet E should have been 0.27% Ca and 0.48% nPP. The lowest Ca that could be formulated and mixed for based on the analysis of the basal diets was 0.42%, which was very different from the other 2 lowest Ca Trt (0.27% in both Trt A and I). Therefore, Ca concentrations used in the factorial analysis were 0.51% (Trt B, F, and J), 0.77% (Trt C, G, and K), and 1.02% (Trt D, H, and L). Tukey's adjustment was applied in all pair-wise comparisons to protect *P*-values. Significance was declared at $P < 0.05$. All regressions were generated based on pen average (8 pen/Trt), whereas only treatment means and SD are presented in figures.

RESULTS

The analyzed Ca and P concentrations for diets are shown in Table 2. Although the formulated concentrations were used to describe the treatments, all regressions were generated based on analyzed Ca and determined nPP concentrations. In the 3 × 3 factorial analysis, formulated values were used to determine the effects of Ca concentration and supply strategy.

Ca Intake

When Ca from large particle size limestone was provided in a separate feeder, Ca intake from limestone ($R^2 = 0.39$) and total Ca intake ($R^2 = 0.81$) were linearly correlated with the Ca concentration in the crumble diet (Figure 1, $P < 0.05$). Birds fed the crumbled diet containing 0.27% Ca consumed more Ca from the separate limestone source (2.38 g of Ca/bird) than birds fed the crumbled diet with 1.02% Ca (1.19 g of Ca/bird, $P < 0.05$). There was no difference in Ca intake from the limestone in the separate feeder when birds were fed crumbled diets with either 0.51 or 0.77% Ca. Total Ca intake, regardless of source, increased as crumble diet Ca concentration increased from 0.27 to 1.02% (Table 3, $P < 0.05$).

When the large particle size limestone was mixed with the crumble diet, total Ca intake was not affected by the Ca concentration in the crumble part of the diet in birds fed 0.28% nPP diets (Table 3). At higher nPP concentration, total Ca intake was higher ($P < 0.05$) in birds fed diet containing 0.51% crumble Ca (6.93 g/bird) than birds fed diets with the 0.41% (5.96 g/bird) and 1.02% (6.04 g/bird) crumble Ca diet, but similar ($P > 0.05$) to birds fed with the 0.77% crumble Ca diet.

Performance

Overall, BWG and FCR were improved with increasing Ca concentration in the crumble but FI was not affected (Table 4). Body weight gain and FI were 531.3 and 719.4 g/bird, respectively, in birds fed diets containing 0.48% nPP with large particle size limestone added postcrumble and these were the highest ($P < 0.05$) for all treatments. Body weight gain was 510.5 and 509.1 g/bird, respectively, in birds fed 0.28% nPP diets with large particle size limestone added postcrumble or provided separately ($P > 0.05$). Birds consumed 17 g/bird more when large particle size limestone was added postcrumble (697.3 g/bird) than the limestone was provided separately (680.2 g/bird, $P < 0.05$). Feed conversion ratio was not affected by the Ca supply strategy ($P > 0.05$).

When large particle size limestone was added postcrumble, the increase in crumble Ca concentration had no effect on BWG, FI, or FCR at either nPP concentration. The average BWG, FI, and FCR were 511.2 g/bird, 698.3 g/bird, and 1.372, respectively, in birds fed 0.28% nPP diets and 527.1 g/bird, 715.6 g/bird, and

Table 2. Calculated and analyzed Ca and P concentrations in final diets

Item	Treatment											
	A	B	C	D	E	F	G	H	I	J	K	L
Ingredient, % (as is)												
Basal	96.7	96.7	96.7	96.7	96.7	96.7	96.7	96.7	96.7	96.7	96.7	96.7
Limestone #20[1]	0.02	0.70	1.39	2.07	0.00	0.28	0.96	1.64	0.02	0.70	1.39	2.07
Monocalcium phosphate[2]	0.17	0.17	0.17	0.17	1.08	1.08	1.08	1.08	0.17	0.17	0.17	0.17
Chromic oxide (Cr_2O_3)[3]	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Celite[4]	2.71	2.03	1.34	0.66	1.82	1.54	0.86	0.18	2.71	2.03	1.34	0.66
Formulated nutrient concentrations in diet, % (crumbles; as is)												
Total P (tP)	0.58	0.58	0.58	0.58	0.78	0.78	0.78	0.78	0.58	0.58	0.58	0.58
Phytate P (PP)	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Nonphytate P (nPP)	0.28	0.28	0.28	0.28	0.48	0.48	0.48	0.48	0.28	0.28	0.28	0.28
Ca	0.27	0.51	0.77	1.02	0.41	0.51	0.77	1.02	0.27	0.51	0.77	1.02
Analyzed nutrient concentrations in diet, % (crumbles; as is)												
tP	0.51	0.51	0.53	0.52	0.74	0.74	0.74	0.74	0.51	0.51	0.53	0.52
PP[5]	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31
nPP[6]	0.20	0.20	0.22	0.21	0.43	0.43	0.43	0.43	0.20	0.20	0.22	0.21
Analyzed Ca in crumbled diet	0.35	0.48	0.69	0.92	0.42	0.45	0.78	0.86	0.35	0.48	0.69	0.92
Limestone #12 added to crumbled diet,[7] %	0.57	0.44	0.23	0.00	0.50	0.47	0.14	0.06	Ad libitum	Ad libitum	Ad libitum	Ad libitum
Total calculated Ca in diet	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92				

[1]Defined as small particle size limestone (IMI Cal Pro, IN). Analyzed Ca, 36.65%. Particle size, mean diameter (d_{gw}) = 0.402 mm; geometric SD (S_{gw}) = 0.255 mm. Distribution: <0.075 mm; 1.72%; 0.075 to 0.150 mm, 4.99%; 0.150 to 0.250 mm, 10.20%; 0.250 to 0.300 mm, 7.88%; 0.300 to 0.355 mm, 9.36%; 0.355 to 0.425 mm, 13.10%; 0.425 to 0.500 mm, 10.84%; 0.500 to 0.600 mm, 15.96%; 0.600 to 0.710 mm, 9.74%; 0.710 to 0.850 mm, 5.36%; 0.850 to 1.000 mm, 5.36%; >1.000 mm, 0.77%.

[2]Monocal, Kirby Agri, Mechanicsville, MD. Analyzed Ca and P: 16.06 and 21.95%, respectively. Mean diameter (d_{gw}) = 0.759 mm; geometric SD (S_{gw}) = 0.258 mm. Distribution: <0.075 mm, 0.34%; 0.075 to 0.250 mm, 0.90%; 0.250 to 0.300 mm, 0.27%; 0.300 to 0.500 mm, 3.81%; 0.500 to 0.600 mm, 9.13%; 0.600 to 0.710 mm, 17.22%; 0.710 to 0.850 mm, 24.04%; 0.850 to 1.000 mm, 34.83%; 1.000 to 1.180 mm; 8.64%; >1.180 mm, 0.82%.

[3]Chromic oxide (Powder/Technical), Fisher Chemical, Fisher, NJ.

[4]Celite (Food Chemicals Codex grade, Celite Corp., Lompar, CA) used as a filler in the complete diets.

[5]Calculated as analyzed percent PP in basal × 96.7% (inclusion of basal in all final diets).

[6]Concentration determined based on analyzed tP minus analyzed PP.

[7]Defined as large particle size limestone (IMI Cal Pro). Analyzed Ca, 38.60%. Particle size, mean diameter (d_{gw}) = 1.988 mm; geometric SD (S_{gw}) = 1.651 mm. Distribution: <0.075 mm, 0.67%; 0.075 to 0.250 mm, 3.78%; 0.250 to 0.500 mm, 2.61%; 0.500 to 0.850 mm, 1.37%; 0.850 to 1.000 mm, 0.70%; 1.000 to 1.180 mm, 0.09%; 1.180 to 1.400 mm, 2.34%; 1.400 to 1.700 mm, 8.14%; 1.700 to 2.000 mm, 11.08%; 2.000 to 2.360 mm, 12.22%; 2.360 to 2.800 mm, 21.63%; 2.800 to 3.350 mm, 26.98%; >3.350 mm, 7.57%.

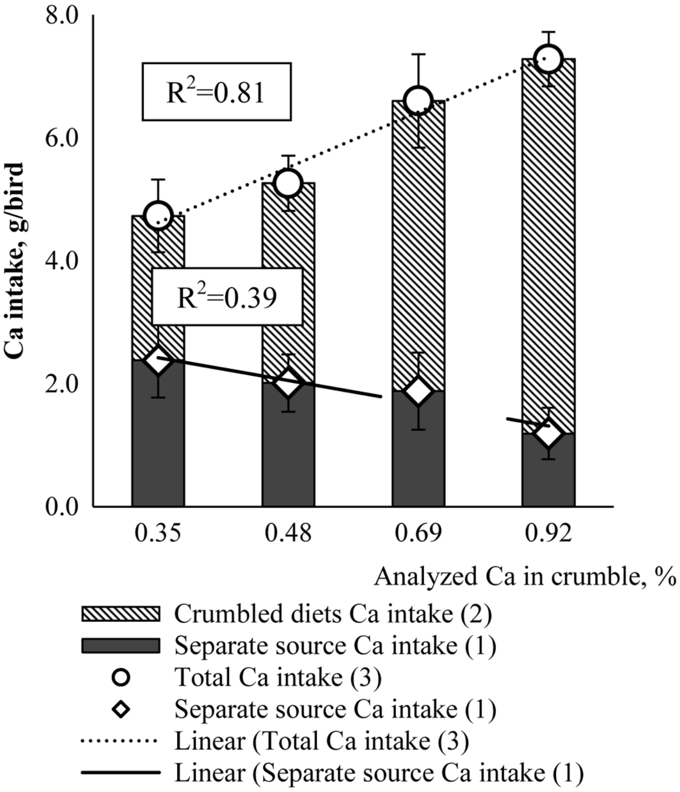

Figure 1. Calcium intake from separate Ca source with low nonphytate P concentration (0.29%) when large particle size limestone was provided separately (mean ± SD). (1) Separate source Ca intake: Ca intake large particle size limestone placed in a separate feeder and offered for ad libitum consumption ($R^2 = 0.39$). (2) Crumbled diets Ca intake: Ca intake from crumbled feed. (3) Total Ca intake: sum of the Ca intake from crumbled feed plus Ca intake from separate feeder limestone ($R^2 = 0.81$).

1.361, respectively, in birds fed 0.48% nPP diets ($P >$ 0.05). When birds were fed diets containing 0.28% nPP and large particle size limestone was provided separately, BWG increased with an improved FCR when crumble Ca concentration increased from 0.27 to 1.02% ($P < 0.05$).

Apparent Ileal Ca and P Digestibilities

There were effects of the crumble diet Ca concentration, Ca supply strategy, and their interaction on AIDCa and AIDP (Table 5, $P < 0.05$). Increased crumble diet Ca concentration led to reduced AIDCa and AIDP and the digestible Ca or P intake ($P < 0.05$). The highest AIDCa (64.79%) was observed when large particle size limestone was offered in a separate feeder, whereas the highest AIDP (50.02%) was observed in birds fed diets containing 0.48% nPP with large particle size limestone added postcrumble ($P < 0.05$).

When the large particle size limestone was provided separately, AIDCa and AIDP in broilers fed the 1.02% crumble Ca diet were negatively affected by crumble Ca concentration compared with birds fed the other 3 diets (Figure 2, $P < 0.05$) that had lower Ca concentration in the crumble. The AIDCa was 68.76, 70.28, 67.10, and 56.99% and AIDP was 62.24, 50.00, 50.53, and 33.22% when crumble Ca was 0.27, 0.51, 0.77, and 1.02%, respectively ($P < 0.05$). In low nPP Trt, the AIDCa was 59.84, 60.76, 54.59, and 49.97% in birds fed diets containing 0.27, 0.51, 0.77, and 1.02% crumble Ca ($P < 0.05$), respectively, with no difference in AIDP (average 39.79%) when large particle size limestone was added to the diets postcrumble (Figure 3A, $P > 0.05$).

When birds were fed 0.48% nPP diets, Ca exerted a strong negative influence on both AIDCa and AIDP (Figure 3B, $P < 0.05$). The AIDCa in birds fed 0.41% Ca diet was 60.24 or 153.5% greater than for birds fed 1.02% crumble Ca diets ($P < 0.05$) containing 0.48% nPP. The AIDP of the 0.48% nPP diets was 65.50, 57.41, 47.89, and 44.78% in birds fed the crumble diets containing 0.41, 0.51, 0.77, and 1.02% Ca, respectively ($P < 0.05$). All the digestible Ca and P intake followed the same pattern as Ca and P digestibility.

Bone Mineralization

Bone mineralization expressed on a percentage of dry defatted bone weight basis was not affected by crumble Ca concentration (Table 6), whereas greater toe and tibia ash weight was found when diets containing higher crumble Ca were fed ($P < 0.05$). Concentration of nPP had a greater effect on bone mineralization when large particle size limestone was supplied separately. Both tibia (865.8 mg/bird, 46.56%) and toe (121.8 mg/bird, 13.80%) ash were greater when 0.48% nPP diets were fed than when the lower nPP diets ($P < 0.05$) were fed. The Ca supply strategy had no effect on bone mineralization when birds were fed low nPP diets. In these low nPP diets, average tibia and toe ash in birds fed diets with low nPP concentration were 722.5 and 102.96 mg/bird when large particle size limestone was mixed with the crumble and 713.1 and 104.6 mg/bird when large particle size limestone was added postcrumble and provided separately, respectively ($P > 0.05$).

DISCUSSION

The ability of chicks to select limestone from a separate feeder to achieve a Ca intake close to requirements is not as clearly seen in the present study as compared with Wilkinson et al. (2014). Wilkinson et al. (2014) demonstrated that broilers have an ability to select a similar Ca intake, of an average of 0.57 g/d, between hatch and 21 d of age when a diet with differing Ca concentrations was offered and large particle size limestone was provided free choice.

In the present work, when a large particle size limestone was offered for ad libitum consumption (Trt I–L), birds increased their intake of the separate Ca source as Ca in the crumble diet decreased. This suggests that birds consumed the large particle size limestone in the separate feeder to a greater degree when the crumbled diets had lower Ca concentrations, which supports the

Table 3. Effect of different nonphytate P (nPP) and Ca added postcrumble or provided separately from the crumbled diets[1] containing varying Ca concentrations on broiler Ca intake from 10 to 20 d of age (n = 8, 8 birds per pen)

Item	Ca,[2] %				nPP,[3] %		True Ca intake, g/bird		Ca intake as a percent of total FI,[6] %	g of BWG/g of Ca intake, %
	FML	Anal.	Added	Total	FML	Det.	From limestone[4]	Total[5]		
Treatment (Trt)										
A	0.27	0.35	0.57	0.92	0.28	0.20	—	6.32[bc]	0.88[bcd]	81.28[cde]
B	0.51	0.48	0.44	0.92	0.28	0.20	—	6.23[c]	0.91[bcd]	80.96[cde]
C	0.77	0.69	0.23	0.92	0.28	0.22	—	6.48[bc]	0.92[bcd]	81.44[cde]
D	1.02	0.92	0.00	0.92	0.28	0.21	—	6.49[bc]	0.92[bc]	79.78[cde]
E	0.41[7]	0.42	0.50	0.92	0.48	0.43	—	5.96[c]	0.85[cde]	86.49[bcd]
F	0.51	0.45	0.47	0.92	0.48	0.43	—	6.93[ab]	0.97[b]	76.09[de]
G	0.77	0.78	0.14	0.92	0.48	0.43	—	6.59[bc]	0.92[bcd]	80.96[cde]
H	1.02	0.86	0.06	0.92	0.48	0.43	—	6.04[c]	0.83[de]	88.60[bc]
I	0.27	0.35	Ad libitum		0.28	0.20	2.38[a]	4.73[d]	0.69[f]	102.78[a]
J	0.51	0.48	Ad libitum		0.28	0.20	2.01[ab]	5.26[d]	0.77[ef]	96.48[ab]
K	0.77	0.69	Ad libitum		0.28	0.22	1.88[ab]	6.60[bc]	0.95[b]	76.18[de]
L	1.02	0.92	Ad libitum		0.28	0.21	1.19[b]	7.28[a]	1.10[a]	70.84[e]
SEM							0.231	0.133	0.019	2.317
P-value							<0.0001	<0.0001	<0.0001	<0.0001
Main effect mean										
Ca concentration,[8] %										
0.51 (Trt B, F, and J)							—	6.14[b]	0.88[b]	84.51[a]
0.77 (Trt C, G, and K)							—	6.56[a]	0.93[a]	79.53[b]
1.02 (Trt D, H, and L)							—	6.60[a]	0.95[a]	79.74[b]
Ca supply strategy										
Postcrumble, low nPP							—	6.40	0.92	80.71
Postcrumble, high nPP							—	6.52	0.91	81.88
Separate feeder, low nPP							—	6.38	0.94	81.16
P-value										
Ca concentration							—	0.0001	<0.0001	0.0080
Ca supply strategy							—	0.4026	0.0887	0.7940
Ca concentration × Ca supply strategy							—	<0.0001	<0.0001	<0.0001

[a–f]Least squares means within a column with different superscript letters differ ($P < 0.05$).

[1]Large particle size limestone provided in a separate feeder from that of the crumbled diets.

[2]FML: formulated concentrations in crumbled diets; Anal.: analyzed concentrations in crumbled diets; Added: large particle size limestone (38.6% Ca) mixed postcrumble, added by weight based on analyzed Ca in both crumbled diets and limestone; Total: calculated concentrations, sum of analyzed Ca in crumbled diets and added Ca from limestone mixed postcrumble; Ad libitum: Ca as large particle size limestone provided in a separate feeder for ad libitum consumption.

[3]FML: formulated nPP concentrations; Det.: determined nPP concentrations based on the difference between analyzed total P and analyzed phytate P concentration.

[4]Ca intake from large particle size limestone provided in a separate feeder for ad libitum consumption. Ca (g/bird) = weight of limestone grit × 38.60%.

[5]Total: 1) for limestone provided in a separate feeder for ad libitum consumption, sum of the Ca intake from crumbled diets plus Ca intake from limestone and 2) for treatments where limestone was mixed postcrumble, weight of diet offered (crumble + limestone) × Ca in in final feed + (limestone + leftover diet + waste in excreta pan) × Ca in leftovers and waste.

[6]Percent Ca intake as a percent of total feed intake (FI) = total true Ca intake (g/bird)/total FI (g/bird) × 100%.

[7]Because the basal diet Ca was analyzed at 0.24% and to achieve the nPP concentration desired of 0.48% with monocalcium phosphate, the minimum Ca concentration that could be achieved was 0.41% rather than the 0.27% Ca desired.

[8]Factorial analysis was performed based on formulated concentrations. The treatments with lowest Ca concentration from each Ca supply strategy were excluded from factorial analysis.

Table 4. Effect of different nonphytate P (nPP) and Ca added postcrumble or provided separately from the crumbled diets[1] containing varying Ca concentrations on broiler growth performance from 10 to 20 d of age (n = 8, 8 birds/pen)

Item	Ca,[2] %				nPP,[3] %		Final BW,[4] g/bird	BWG,[5] g/bird	FI,[5] g/bird	FCR[5]
	FML	Anal.	Total	True	FML	Det.				
Treatment (Trt)										
A	0.27	0.35	0.92	0.88	0.28	0.20	780.8^{ab}	513.2^{abc}	701.0^{abc}	1.385^{ab}
B	0.51	0.48	0.92	0.91	0.28	0.20	771.6^{abc}	503.8^{bcd}	687.7^{bcd}	1.366^{ab}
C	0.77	0.69	0.92	0.92	0.28	0.22	778.1^{ab}	510.4^{abc}	701.6^{abc}	1.376^{ab}
D	1.02	0.92	0.92	0.92	0.28	0.21	785.3^{ab}	517.3^{abc}	702.7^{abc}	1.359^{b}
E	0.41[6]	0.42	0.92	0.85	0.48	0.43	782.3^{ab}	514.5^{abc}	704.2^{abc}	1.369^{ab}
F	0.51	0.45	0.92	0.97	0.48	0.43	794.2^{ab}	526.9^{abc}	714.0^{abc}	1.380^{ab}
G	0.77	0.78	0.92	0.92	0.48	0.43	800.0^{a}	532.6^{ab}	718.9^{ab}	1.336^{bc}
H	1.02	0.86	0.92	0.83	0.48	0.43	802.0^{a}	534.4^{a}	725.2^{a}	1.358^{b}
I	0.27	Ad libitum		0.69	0.28	0.20	747.0^{c}	479.3^{d}	682.0^{bcd}	1.424^{a}
J	0.51	Ad libitum		0.77	0.28	0.20	766.0^{bc}	498.4^{cd}	681.2^{cd}	1.368^{ab}
K	0.77	Ad libitum		0.95	0.28	0.22	782.0^{ab}	514.4^{abc}	696.4^{abcd}	1.355^{bc}
L	1.02	Ad libitum		1.10	0.28	0.21	781.8^{ab}	514.4^{abc}	663.2^{d}	1.290^{c}
SEM							6.36	6.34	7.78	0.0137
P-value							<0.0001	<0.0001	<0.0001	<0.0001
Main effect mean										
Ca concentration,[7] %										
0.51 (Trt B, F, and J)							777.3	509.7^{b}	694.3	1.371^{b}
0.77 (Trt C, G, and K)							786.7	519.1^{ab}	705.6	1.356^{ab}
1.02 (Trt D, H, and L)							789.7	522.1^{a}	697.0	1.335^{a}
Ca supply strategy										
Postcrumble, low nPP (Trt B–D)							778.3^{b}	510.5^{b}	697.3	1.367
Postcrumble, high nPP (Trt F–H)							798.7^{a}	531.3^{a}	719.4	1.358
Separate feeder, low nPP (Trt J–L)							776.6^{b}	509.1^{b}	680.2	1.338
P-value										
Ca concentration							0.0555	0.0401	0.1762	0.0219
Ca supply strategy							<0.0001	<0.0001	<0.0001	0.0623
Ca concentration × Ca supply strategy							0.9014	0.8825	0.0674	0.0342

[a–d]Least squares means within a column with different superscript letters differ ($P < 0.05$).

[1]Large particle size limestone provided in a separate feeder from that of the crumbled diets.

[2]FML: formulated concentrations in crumbled diets; Anal.: analyzed concentrations in crumbled diets; Added: large particle size limestone (38.6% Ca) mixed postcrumble, added by weight based on analyzed Ca in both crumbled diets and limestone; Total: calculated concentrations, sum of analyzed Ca in crumbled diets and added Ca from limestone mixed postcrumble; True: true Ca% consumed by birds (Table 3); Ad libitum: Ca as large particle size limestone provided in a separate feeder for ad libitum consumption.

[3]FML: formulated nPP concentrations; Det.: determined nPP concentrations based on the difference between analyzed total P and analyzed phytate P concentration.

[4]Initial BW = 267.7 ± 0.22 (SEM) g/bird ($P = 0.78$).

[5]Body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were corrected by mortalities.

[6]Because the basal diet Ca was analyzed at 0.24% and to achieve the nPP concentration desired of 0.48% with monocalcium phosphate, the minimum Ca concentration that could be achieved was 0.41% rather than the 0.27% Ca desired.

[7]Factorial analysis was performed based on formulated concentrations. The treatments with lowest Ca concentration from each Ca supply strategy were excluded from factorial analysis.

Table 5. Effect of different nonphytate P (nPP) and Ca added postcrumble or provided separately from the crumbled diets[1] containing varying Ca concentrations on broiler apparent ileal Ca and P digestibility at 20 d of age (n = 8, 8 birds per replicate)

	Ca,[2] %				nPP,[3] %		Digestibility, %		Digestible intake, g/bird		Digestible intake, mg/g of BWG	
Item	FML	Anal	Total	True	FML	True	Ca	P[4]	Ca	P	Ca	P
Treatment (Trt)												
A	0.27	0.35	0.92	0.88	0.28	0.20	59.84^{abcd}	41.75^{cdef}	3.78^{bc}	1.67^{ef}	7.38^{bc}	3.25^{e}
B	0.51	0.48	0.92	0.91	0.28	0.20	60.76^{abc}	35.34^{ef}	3.77^{bc}	1.30^{f}	7.51^{abc}	2.58^{e}
C	0.77	0.69	0.92	0.92	0.28	0.22	54.59^{cd}	42.52^{cdef}	3.41^{bcd}	1.67^{ef}	6.66^{bcd}	3.28^{e}
D	1.02	0.92	0.92	0.92	0.28	0.21	49.97^{de}	39.54^{def}	3.24^{cd}	1.59^{ef}	6.25^{cd}	3.06^{e}
E	0.41[5]	0.42	0.92	0.85	0.48	0.43	60.24^{abcd}	65.50^{a}	3.58^{bc}	3.72^{a}	6.96^{bc}	7.22^{a}
F	0.51	0.45	0.92	0.97	0.48	0.43	57.62^{bcd}	57.41^{ab}	3.98^{abc}	3.19^{b}	7.53^{abc}	6.05^{b}
G	0.77	0.78	0.92	0.92	0.48	0.43	41.42^{e}	47.89^{cd}	2.69^{d}	2.66^{c}	5.04^{d}	5.00^{c}
H	1.02	0.86	0.92	0.83	0.48	0.43	23.76^{f}	44.78^{cde}	1.40^{e}	2.59^{c}	2.62^{e}	4.84^{c}
I	0.27	Ad libitum		0.69	0.28	0.20	68.76^{a}	62.24^{a}	3.23^{cd}	2.36^{cd}	6.75^{bc}	4.66^{cd}
J	0.51	Ad libitum		0.77	0.28	0.20	70.28^{a}	50.00^{bc}	3.66^{bc}	1.89^{de}	7.25^{bc}	3.80^{de}
K	0.77	Ad libitum		0.95	0.28	0.22	67.10^{ab}	50.53^{bc}	4.66^{a}	2.05^{de}	9.07^{a}	3.99^{d}
L	1.02	Ad libitum		1.10	0.28	0.21	56.99^{bcd}	33.22^{f}	4.14^{ab}	1.26^{f}	8.14^{ab}	2.48^{e}
SEM							2.240	2.044	0.182	0.102	0.345	0.242
P-value							<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Main effect mean												
Ca concentration,[6] %												
0.51 (Trt B, F, and J)							62.89^{a}	47.59^{a}	3.80^{a}	2.13^{a}	7.43^{a}	4.14^{a}
0.77 (Trt C, G, and K)							54.37^{b}	46.98^{a}	3.59^{a}	2.13^{a}	6.92^{b}	4.09^{a}
1.02 (Trt D, H, and L)							43.58^{c}	39.18^{b}	2.92^{b}	1.81^{b}	5.67^{c}	3.46^{b}
Ca supply strategy												
Postcrumble, low nPP (Trt B–D)							55.11^{b}	39.13^{c}	3.47^{b}	1.52^{c}	6.81^{b}	2.97^{c}
Postcrumble, high nPP (Trt F–H)							40.93^{c}	50.02^{a}	2.69^{c}	2.81^{a}	5.06^{c}	5.30^{a}
Separate feeder, low nPP (Trt J–L)							64.79^{a}	44.58^{b}	4.15^{a}	1.73^{b}	8.15^{a}	3.42^{b}
P-value												
Ca concentration							<0.0001	<0.0001	<0.0001	0.0002	<0.0001	<0.0001
Ca supply strategy							<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Ca concentration × Ca supply strategy							<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a–f]Least squares means within a column with different superscript letters differ ($P < 0.05$).

[1]Large particle size limestone provided in a separate feeder from that of the crumbled diets.

[2]FML: formulated concentrations in crumbled diets; Anal.: analyzed concentrations in crumbled diets; Added: large particle size limestone (38.6% Ca) mixed postcrumble, added by weight based on analyzed Ca in both crumbled diets and limestone; Total: calculated concentrations, sum of analyzed Ca in crumbled diets and added Ca from limestone mixed postcrumble; True: true Ca% consumed by birds (Table 3); Ad libitum: Ca as large particle size limestone provided in a separate feeder for ad libitum consumption.

[3]FML: formulated nPP concentrations; Det.: determined nPP concentrations based on the difference between analyzed total P and analyzed phytate P concentration.

[4]P: total P.

[5]Because the basal diet Ca was analyzed at 0.24% and to achieve the nPP concentration desired of 0.48% with monocalcium phosphate, the minimum Ca concentration that could be achieved was 0.41% rather than the 0.27% Ca desired.

[6]Factorial analysis was performed based on formulated concentrations. The treatments with lowest Ca concentration from each Ca supply strategy were excluded from factorial analysis.

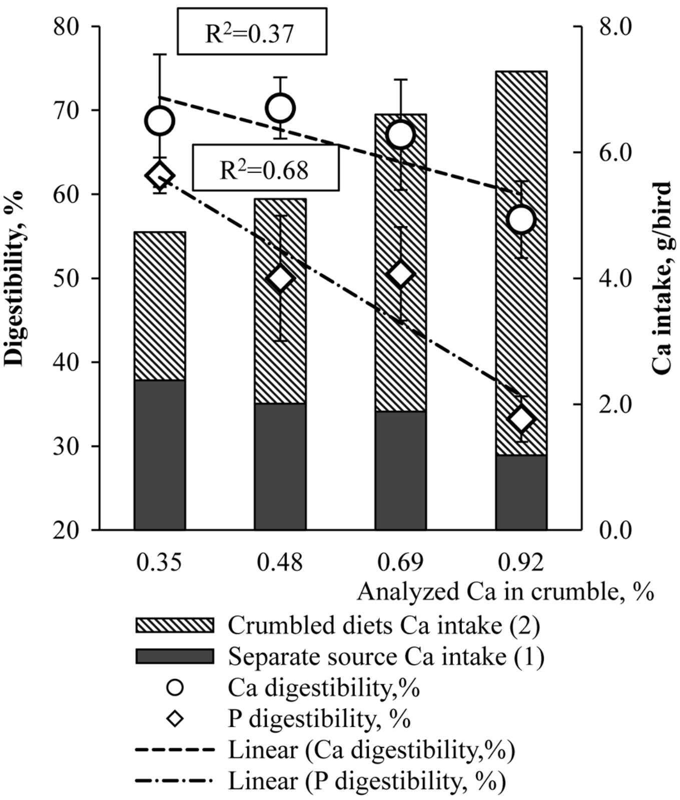

Figure 2. Effect of Ca concentration in the crumbled diet on ileal Ca and P digestibilities when large particle size limestone was provided separately (mean ± SD). (1) Separate source Ca intake: Ca intake from large particle size limestone placed in a separate feeder and offered for ad libitum consumption. (2) Crumbled diets Ca intake: Ca intake from crumbled diets. Total Ca intake: sum of the Ca intake from crumbled diets plus Ca intake from separate feeder limestone.

Wilkinson et al. (2014) results. However, in the current experiment, broilers did not consume sufficient quantities of the separate Ca source to match the Ca intake of birds that were fed the crumble diet with limestone mixed in with the crumble diet (Table 3). Because birds fed the lowest crumble Ca diet ingested less Ca overall, the BWG and tibia ash weight of these birds were lower than those for birds fed the higher (0.77 and 1.02% Ca) Ca crumble diet. This confirms that the birds did not choose to consume limestone from a separate feeder in sufficient quantities to reach or approximate their Ca requirement as had been reported by Wilkinson et al. (2014).

The extensive exploration of Ca specific appetite in poultry done during the 1960s and 1970s, as well as a recent study, have confirmed that poultry have a Ca-specific appetite. Although the Ca-specific appetite has been demonstrated, not all birds respond to Ca deficiency equally (Wilkinson et al., 2014). Birds need to perceive a Ca deficiency as well as associate the organoleptic properties of the separate source limestone with Ca. It would appear that learned behavior may be important for this mechanism (Wood-Gush, 1966; Taher et al., 1984). One major difference between the Wilkinson et al. (2014) study and the current study is that birds were given the experimental diets from hatch in the Wilkinson et al. (2014) study, whereas birds were not fed the experimental diets until d 10 in the current trial. If time is needed for birds to develop an appetite for Ca, to associate organoleptic properties of an ingredient source with a specific nutrient and to learn to compensate for the dietary deficiency by consuming an ingredient with a high Ca concentration, this would

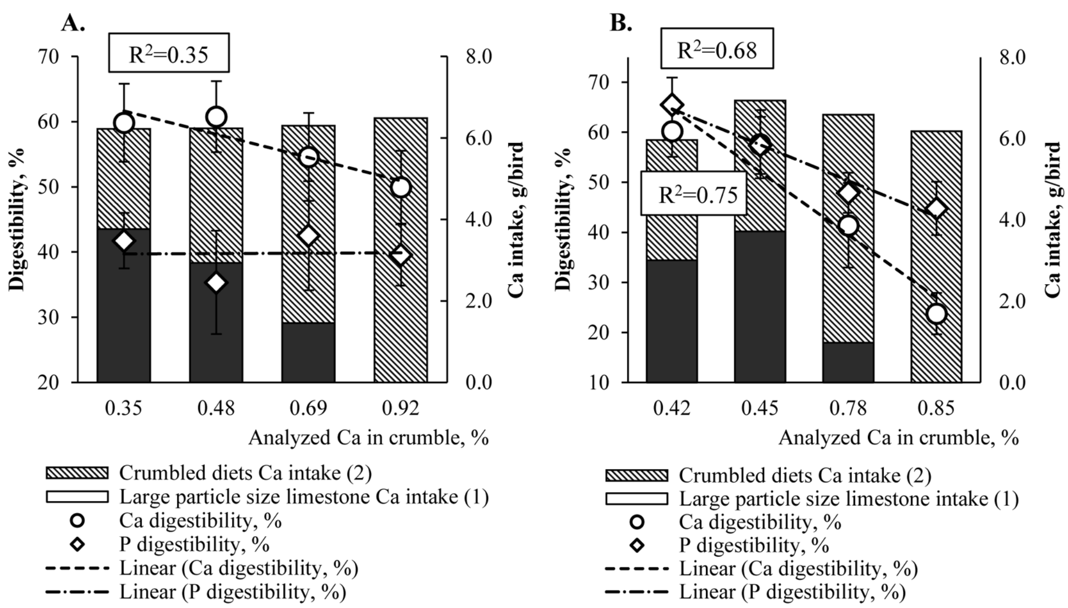

Figure 3. Effect of Ca concentration in the crumbled diets on ileal Ca and P digestibilities when large particle size limestone was mixed with crumbled diets. A. Diets containing 0.29% nonphytate P. B. Diets containing 0.49% nonphytate P (mean ± SD). (1) Large particle size limestone Ca intake, Ca intake from large particle size limestone mixed with crumbled diets. (2) Crumbled diets Ca intake, Ca intake from crumbled diets. The Ca intake from large particle size limestone and crumbled diets was calculated based on the percentage of Ca contributed by each source (crumbled diets or large particle size limestone) in the final diet, assuming the large particle size limestone was distributed evenly in the crumbled diets. The sum of Ca intake from crumbled feed plus Ca intake from separate feeder limestone equals the total Ca intake.

Table 6. Effect of different nonphytate P (nPP) and Ca added postcrumble or provided separately from the crumbled diets[1] containing varying Ca concentrations on broiler bone mineralization at 20 d of age (n = 8, 8 birds per pen)

Item	Ca,[2] %				nPP,[3] %		Tibia ash[4]		Toe[4]	
	FML	Anal	Total	True	FML	Det.	mg/bone	%	mg/bird	%
Treatment (Trt)										
A	0.27	0.35	0.92	0.88	0.28	0.20	703.8^{cd}	42.60^{a}	97.2^{d}	11.55^{b}
B	0.51	0.48	0.92	0.91	0.28	0.20	710.3^{cd}	43.05^{b}	100.7^{cd}	11.71^{b}
C	0.77	0.69	0.92	0.92	0.28	0.22	724.1^{cd}	42.42^{b}	100.6^{cd}	11.67^{b}
D	1.02	0.92	0.92	0.92	0.28	0.21	731.3^{c}	43.10^{b}	107.3^{b}	12.18^{b}
E	0.41[5]	0.42	0.92	0.85	0.48	0.43	832.4^{b}	46.79^{a}	118.2^{a}	13.25^{a}
F	0.51	0.45	0.92	0.97	0.48	0.43	862.7^{ab}	46.85^{a}	120.7^{a}	13.68^{a}
G	0.77	0.78	0.92	0.92	0.48	0.43	858.0^{ab}	46.13^{a}	123.7^{a}	13.87^{a}
H	1.02	0.86	0.92	0.83	0.48	0.43	875.9^{a}	46.71^{a}	121.1^{a}	13.84^{a}
I	0.27	Ad libitum		0.69	0.28	0.20	658.0^{e}	42.41^{b}	103.6^{bc}	12.16^{b}
J	0.51	Ad libitum		0.77	0.28	0.20	688.5^{de}	42.40^{b}	104.9^{bc}	12.20^{b}
K	0.77	Ad libitum		0.95	0.28	0.22	739.0^{c}	42.90^{b}	102.3^{bcd}	12.16^{b}
L	1.02	Ad libitum		1.10	0.28	0.21	710.8^{cd}	42.63^{b}	103.8^{bc}	11.81^{b}
SEM							7.96	0.292	1.23	0.150
P-value							<0.0001	<0.0001	<0.0001	<0.0001
Main effect mean										
Ca concentration,[6] %										
0.51 (Trt B, F, and J)							753.7^{b}	44.10	108.8^{b}	12.53
0.77 (Trt C, G, and K)							774.6^{a}	43.81	108.9^{b}	12.47
1.02 (Trt D, H, and L)							773.1^{a}	44.08	111.6^{a}	12.61
Ca supply strategy										
Postcrumble, low nPP (Trt B–D)							722.5^{b}	42.79^{b}	102.9^{b}	11.85^{b}
Postcrumble, high nPP (Trt F–H)							865.8^{a}	46.56^{a}	121.8^{a}	13.80^{a}
Separate feeder, low nPP (Trt J–L)							713.1^{b}	42.64^{b}	104.6^{b}	12.05^{b}
P-value										
Ca concentration							<0.0001	0.2422	0.0114	0.7992
Ca supply strategy							<0.0001	<0.0001	<0.0001	<0.0001
Ca concentration × Ca supply strategy							<0.0001	0.0764	0.0035	0.0254

[a–e]Least squares means within a column with different superscript letters differ ($P < 0.05$).

[1]Large particle size limestone provided in a separate feeder from that of the crumbled diets.

[2]FML: formulated concentrations in crumbled diets; Anal.: analyzed concentrations in crumbled diets; Added: large particle size limestone (38.6% Ca) mixed postcrumble, added by weight based on analyzed Ca in both crumbled diets and limestone; Total: calculated concentrations, sum of analyzed Ca in crumbled diets and added Ca from limestone mixed postcrumble; True: true Ca% consumed by birds (Table 3); Ad libitum: Ca as large particle size limestone provided in a separate feeder for ad libitum consumption.

[3]FML: formulated nPP concentrations; Det.: determined nPP concentrations based on the difference between analyzed total P and analyzed phytate P concentration.

[4]Dry defatted ash weight per tibia or 2 middle toes (bird) and percent in tibia and toe.

[5]Because the basal diet Ca was analyzed at 0.24% and to achieve the nPP concentration desired of 0.48% with monocalcium phosphate, the minimum Ca concentration that could be achieved was 0.41% rather than the 0.27% Ca desired.

[6]Factorial analysis was performed based on formulated concentrations. The treatments with lowest Ca concentration from each Ca supply strategy were excluded from factorial analysis.

possibly explain why Ca intake from the separate feeder limestone did not meet the birds' requirement in the low crumble Ca Trt (0.27% and 0.51% Ca). In addition, because broiler breeds differed between the 2 studies [Cobb 500 in Wilkinson et al. (2014) vs. Hubbard 99 M × Cobb 500 F in the current study], the variations in response to Ca deficiency could also be partially due to genetic differences.

In the current study the method of supplying large particle size limestone rather than nPP concentration was the major factor that affected Ca intake. When the large particle size limestone was mixed with the diets postcrumble (Trt B–D, F–H), total Ca intake was similar in birds fed similar nPP diets (Table 3). This suggests that even though particle size was different between the crumble diet (d_{gw} = 1.969 mm, S_{gw} = 1.088 mm) and the limestone (d_{gw} = 1.988 mm; S_{gw} = 1.651 mm), the chicks were unable to differentiate between the crumble diet and the limestone when these 2 components were mixed and so consumed limestone and crumbles together essentially as a complete diet. This speculation is supported by performance (Table 4), bone ash (Table 5), and based on total Ca intake calculations based on Ca concentration in the mix diet times the FI (Table 3). Although birds fed 0.48% nPP diets overall had greater BWG and bone ash, the pattern of the birds' response to different Ca concentrations from the crumble diet and added large particle size limestone was very similar to that of birds fed the 0.28% nPP diet (Trt A–D).

Crumble Ca concentration, Ca supply strategy, and their interactions all affected AIDCa (Table 5, Figures 2 and 3). When limestone was supplied in a separate feeder (Trt J–L), Ca digestibility was greater than when limestone was added and mixed postcrumble (Trt B–D, F–H). The greater digestibility can be partially related to the overall lower total Ca intake in birds on Trt where the limestone was supplied in a separate feeder (6.46 vs. 6.38 g/bird). Plumstead et al. (2008) showed a similar trend between dietary Ca concentration and apparent ileal Ca digestibility in broiler chickens. In contrast, Tamim et al. (2004) reported that the AIDCa was 38% higher in birds fed 0.65% Ca diet than 0.17% Ca diet. The difference is probably related to the fact that in the Tamim et al. (2004) study, the low Ca diet had no added limestone or inorganic Ca source. If the diet Ca digestibility would be compared with a higher Ca diet, a greater Ca digestibility would be expected because the Ca bioavailability in limestone is higher than that the Ca in corn and soybean meal (Reid and Weber, 1976; Tamim et al., 2004). Therefore, caution should be taken when comparing such results.

When large particle size limestone was added postcrumble (Trt A–H), despite similar amounts of Ca being consumed among all 8 Trt (6.38 g/bird), the AIDCa was negatively associated with increasing crumble Ca concentration in birds fed similar nPP diets (R^2 = 0.35 and 0.75 in 0.28% and 0.48% nPP, respectively). Although all 8 Trt had a similar dietary Ca concentration, the presence of large particle size and small particle size limestone differed. When crumble Ca increased from 0.27 to 1.02%, the proportion of large particle size limestone dropped from 0.65 to 0%. This suggests that digestibility of Ca was lower in the smaller particle size limestone (d_{gw} = 0.402 mm, S_{gw} = 0.255 mm). These 2 Ca sources came from the same quarry but were not necessarily the same so that the difference in AIDCa could have been due to particle size, source, or both. Similar results were reported where a significant improvement in Ca digestibility was found when a greater percentage of coarse particle size limestone was fed to laying hens (Lichovnikova, 2007; Kim et al., 2014). Several studies have shown that the in vitro solubility of limestone is lower for large particle sizes compared with limestone of smaller particle size (Rao and Roland, 1989; Saunders-Blades et al., 2009; Walk et al., 2012; Kim et al., 2014). In laying hens, the slow release of large particle size limestone prolongs its retention in the gizzard, resulting in improved in vivo solubility defined as the concentration difference of limestone fed and that found in excreta, leading to an improved digestibility in the lower tract (Rao and Roland, 1989).

In birds fed diets containing 0.48% nPP (Trt E–H), Ca digestibility response to changes in composition of large/small particle size limestone was more obvious than that in the 0.28% nPP Trt. The antagonizing effect between Ca and P is well established. Not only can Ca chelate with phytate-P to form insoluble complexes at higher pH (Luttrell, 1993; Angel et al., 2002; Tamim and Angel, 2003), but Ca can also interact with inorganic P, forming precipitates (Hurwitz and Bar, 1971). These interactions of Ca with the different P forms lead to impaired Ca digestibility as Ca intake increases. In addition, smaller particle size limestone dissolves much faster than large particle size limestone (Walk et al., 2012; Kim et al., 2014), creating more opportunity for the Ca to interact with P. This may explain the low Ca digestibility in the 0.48% nPP Trt and the steeper drop in Ca digestibility when the proportion of large particle size limestone decreased.

Similar to the AIDCa, AIDP was affected by Ca concentration in the crumble diet and limestone supply strategy, except in birds fed the 0.28% nPP diets (Trt A–D). Several studies have shown that inorganic Ca complexes with phytic acid or it can precipitate with inorganic P, reducing P availability (Hurwitz and Bar, 1971; Luttrell, 1993; Angel et al., 2002; Tamim and Angel, 2003). It is unclear why AIDP was not affected in birds fed 0.28% nPP when large particle size limestone was added postcrumble, but it is possible that the potential benefit from feeding large particle size limestone was offset by the overall imbalanced Ca:nPP ratio (4.40:1, as consumed) in the diet.

When birds were fed the 0.28% nPP diet with the large particle size limestone provided in a separate feeder, AIDP was reduced as crumble diet Ca concentration increased (Trt I–L). This effect can be related to 1) the increased total Ca intake from 4.73 to 7.28 g/bird as shown in several studies (Mohammed et al., 1991; Tamim et al., 2004; Plumstead et al., 2008), 2) the reduced proportion of large particle size limestone in the diet, or 3) both.

In general, feeding a high (0.48%) nPP diet (Trt F-H) improved AIDP by 28% compared with that of birds fed the low (0.28%) nPP diet (Trt B-D) when large particle size limestone was added postcrumble. The 0.48% nPP diets were more balanced (Ca and nPP ratio of 2.07:1 as consumed) compared with that in the 0.28% nPP diets (4.32:1). As large limestone particle size in the diet increased and small particle limestone decreased, with changes in Ca concentration in the crumbled diet, AIDP increased and this increase reflects that seen in AIDCa.

Wilkinson et al. (2014) found that broilers were able to chose Ca when limestone was fed in a separate feeder, such that total Ca intake was similar or above their requirement. They also reported that feeding a Ca-deficient diet and providing a Ca source in a separate feeder improved both AIDCa and AIDP compared with those in birds fed diets with Ca concentration close to requirements. The current experiment was designed, in part, to confirm the findings reported previously by Wilkinson et al. (2014). It is unclear what size limestone was used in the complete diets fed by Wilkinson et al. (2014), but the particle size limestone used in the separate feeders was similar in both experiments. In the current trial, broilers were unable to choose enough Ca from a separate feeder Ca source to reach their Ca requirements or reach the Ca concentrations consumed by the birds fed the higher crumbled Ca diet and this is in contrast to the findings reported by Wilkinson et al. (2014). Thus, although an improved AIDP was observed in the lowest Ca concentration crumbled diet with large particle size limestone provided separately (Trt I), this benefit was possibly related to the low total Ca intake.

Phosphorus is a limited resource and is the third most costly ingredient in poultry diets. Improving the utilization of P in poultry diets will decrease the amount of inorganic P needed, reduce P excretion and feed costs, as well as increase the sustainability of broiler production. Despite the previous work by Wilkinson et al. (2014), our study found that the Ca-specific appetite in broilers was not sufficient when containing less than 0.77% Ca in the crumble or that we did not allow sufficient time for the birds to adapt for them to meet their requirement. In addition, when the large particle size limestone was mixed with the crumbled diet, the AIDP improvement seen when a separate Ca source was fed, disappeared. However, in nPP-sufficient diets, the benefit of low Ca crumbled diets is clear, an effect that has promising implications. Future research may need to validate this strategy in work that looks also at P and Ca retention, and the time it takes for the birds to adapt their feeding behavior, as economic implications and the potential benefits in feed manufacturing costs.

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