

# Determination of the standardized ileal digestible calcium requirement of male Arbor Acres Plus broilers from hatch to day 10 post-hatch

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**ABSTRACT** Arbor Acres Plus male broilers (n = 1,152) were obtained at hatch and allocated to 1 of 6 diets from hatch to d 10 post-hatch. There were 16 replicate cages per diet and 12 birds per cage. Five of the diets were formulated to contain graded concentrations of standardized ileal digestible (SID) Ca at 0.60, 0.50, 0.40, 0.30, or 0.20%. A sixth reference diet was formulated using total Ca coefficients for each ingredient and contained 0.96% total Ca. Available P (avP) was maintained at 0.48% in all 6 diets. Data were subjected to an analysis of variance and the model included diet and block. Means were separated using contrasts to determine linear or quadratic effects of SID Ca and using the Dunnett's test to compare the reference diet to all SID Ca diets. There was no effect of graded levels of SID Ca on intake or gain. Birds fed diets containing 0.60, 0.50, 0.30, or 0.20% SID Ca ate ( $P < 0.05$ ) or gained ( $P < 0.05$ ) more compared with birds fed the reference diet. Mortality corrected FCR improved (linear,  $P < 0.05$ ) as

the SID Ca concentration in the diet increased from 0.20 to 0.60%. Tibia ash percent was greatest in birds fed 0.50% SID Ca and lowest in birds fed 0.20% SID Ca (quadratic,  $P < 0.05$ ). Tibia ash percent was lower ( $P < 0.05$ ) in birds fed diets formulated to contain 0.20% SID Ca compared with birds fed the reference diet. No other differences in tibia ash were reported. Apparent ileal digestibility (AID) or retention of P was greater ( $P < 0.05$ ) in birds fed diets formulated using SID Ca compared with birds fed the reference diet. The AID of P increased (linear,  $P < 0.05$ ) as the SID Ca content in the diet decreased from 0.60 to 0.20%. The AID or retention of Ca was similar in birds fed 0.60 or 0.50% SID Ca and increased as SID Ca decreased to 0.20% (quadratic,  $P < 0.05$ ). Regression equations developed using bone ash and apparent P retention estimate the SID Ca requirement of Arbor Acres Plus broilers from hatch to d 10 post-hatch was 0.53 and 0.49%, respectively. This corresponds to a SID Ca to available P ratio of 1.1 to 1.02.

**Key words:** amino acid, broiler, digestible calcium, phosphorus, phytate-free

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## INTRODUCTION

Calcium and P are the most abundant minerals in the body and are predominantly found in the skeleton. Deficiencies of Ca or P or an imbalanced Ca to P ratio result in reduced growth performance, feed efficiency, bone mineralization, and may even lead to death in severe cases. However, excess dietary Ca can negatively impact feed efficiency and P and amino acid digestibility (Amerah et al., 2014). Understanding the amount of dietary Ca and P that are digested, absorbed, and used by the animal is vitally important for bird welfare

(Angel, 2017), including skeletal and gut health (Paiva et al., 2013, 2014) and efficient use of resources, such as inorganic P.

Poultry diets are currently formulated using total Ca and digestible, non-phytate or available P (avP). Considerable efforts have been made to determine the digestible Ca coefficients of limestone, monocalcium phosphate, dicalcium phosphate, and meat and bone meal for broilers (Anwar et al., 2015, 2016<sup>a,b,c</sup>, 2017, 2018; Zhang and Adeola, 2018; David et al., 2019; Kim et al., 2019). Unfortunately, a standard method to determine digestible Ca coefficients for feed ingredients is not currently available and numerous experimental methods have been employed. These include differences in the basal diet (corn or semipurified), Ca or P concentration or ratios, adaptation time the birds are fed the experimental diets, the presence or absence of phytase, and evaluation of different limestone sources and particle sizes. However, combining the current information of

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**Table 1.** Calculated standardized ileal digestible calcium coefficients of various feed ingredients.

Ingredient	Total calcium, %	Standardized ileal digestible (SID) calcium coefficient <sup>1</sup>
Wheat	0.04	0.71
Corn dried distillers grains with solubles	0.08	0.70
Corn	0.01	0.70
Soybean meal	0.34	0.54
Canola meal	0.72	0.31
Limestone <sup>2</sup>	37.86	0.56
Dicalcium phosphate	22.41	0.42

<sup>1</sup>Mean values presented in [Walk et al., \(2021\)](#).

<sup>2</sup>Limestone particle size and in vitro solubility were determined and the SID Ca coefficient was estimated using methods of [Kim et al. \(2019\)](#).

the digestible Ca coefficients for the different feed ingredients may provide information and guidance for future developments of a digestible Ca system for broilers. Recently, [Walk et al. \(2021\)](#) presented the mean, minimum and maximum standardized ileal digestible (SID) Ca coefficients for a few commonly used feed ingredients. Some of these coefficients were then used in the current study ([Table 1](#)) to determine the SID Ca requirement of fast-growing broilers from hatch to d 10 post-hatch. Due to the impact of particle size and phytase on the calcium digestibility of limestone ([Kim et al., 2018](#)), the SID Ca coefficient for the limestone used in the current study was estimated using particle size, in vitro solubility at 15 and 30 min, and an equation from [Kim et al. \(2019\)](#). Furthermore, because dietary phytate can impact Ca digestibility, phytase was supplemented at levels expected to create nearly 'phytate-free diets' in the current experiment ([Cowieson et al., 2017](#); [Walk and Olu-kosi, 2019](#)).

## MATERIALS AND METHODS

The animal protocol for this research was approved by the Animal Welfare Committee of DSM (China) Animal Nutrition Research Center and complied with the guidelines in the European Union council directive 2010/63/EU for animal experiments. This experiment was conducted at DSM (China) Animal Nutrition Research Center Co., Ltd (Bazhou, P. R. China).

One thousand one hundred and fifty two Arbor Acres Plus male broilers were obtained on day of hatch and randomly allocated to 1 of 6 experimental diets in battery cages. There were 12 birds per cage from hatch to d 10 post-hatch. Each diet was fed to 16 replicate cages. All birds were reared in cages in an environmentally controlled room with a lighting program of 23L:1D during the first week and 20L:4D afterward until the end of the trial. The temperature of the room was adjusted according to breed guidelines. Birds were allowed ad libitum access to feed and water.

Dietary treatments consisted 5 diets formulated to contain graded levels of SID Ca at 0.60, 0.50, 0.40, 0.30, or 0.20%. The ingredient SID Ca coefficients used to

create the graded concentrations of SID Ca are listed in [Table 1](#). The diets were formulated to meet or exceed all nutrient requirements for fast growing broilers, with the exception of Ca. To mitigate any confounding effects of dietary phytate on Ca digestibility, the SID Ca diets were formulated to contain 3,000 FYT/kg phytase (Ronozyme HiPhos GT, DSM Nutritional Products, Kaiseraugst, Switzerland) at the expense of 0.19% available P. To ensure graded concentrations of SID Ca were achieved, a large batch of the 0.60 and 0.20% SID Ca diets were mixed and then combined at 75:25, 50:50, or 25:75 ratios to create the 0.50, 0.40, or 0.30% SID Ca diets, respectively ([Table 2](#)). The SID Ca diets also contained xylanase (Ronozyme Multigrain, DSM Nutritional Products, Kaiseraugst, Switzerland) and protease (Ronozyme ProAct CT, DSM Nutritional Products, Kaiseraugst, Switzerland) at the expense of energy, protein, and amino acids ([Table 2](#)).

The sixth experimental diet was formulated using total Ca values for all ingredients and contained 0.96% total Ca. This diet served as the reference diet to compare against the SID Ca diets and validate the estimated SID Ca requirements. All nutrients in the reference diet met or exceeded requirements and were equivalent to the nutrients in the SID Ca diets, with the exception of Ca. No exogenous enzymes were added to the reference diet. All 6 diets were formulated to contain 0.48% available P.

Birds were weighed by cage prior to placement (d 0) and d 10 to determine mean BW and calculate mean BW gain (**BWG**). Feed addition and feed left were weighed at d 0 and 10 to calculate feed intake (**FI**). Body weight gain and FI were used to calculate feed conversion ratio (**FCR**). Mortality was recorded daily and any culled or dead birds were weighed. Feed intake and subsequently FCR were adjusted according to the number of bird days per cage.

From d 8 to 10, excreta were collected daily from 12 replicate cages per treatment, pooled within cage, and frozen until further analyses. On d 10 after weighing, all birds in replicate cages 1 to 12 were euthanized by carbon dioxide asphyxiation. Ileal digesta (defined as the Meckel's diverticulum to 40 mm proximal to the ileocecal junction) was collected by flushing with distilled water, pooled within cage, and immediately frozen. Left tibias were obtained from 2 birds of average BW per cage and pooled within cage to determine tibia ash.

Digesta and excreta were freeze dried to a constant weight. Diets, digesta, and excreta were ground to pass a 0.5-mm screen and then analyzed for Ca, P, and Ti using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES; Optima TM 8000, Perkin Elmer, Shelton, CT) after microwave digestion (method 985.01; [AOAC International, 2006](#)). Diets and ileal digesta were analyzed for amino acids using a 24-h hydrolysis in 6 N hydrochloric acid at 110°C under an atmosphere of N. Samples were oxidized in performic acid before acid hydrolysis for methionine and cysteine analyses. Samples for tryptophan analysis were hydrolyzed using barium hydroxide. Amino acids in

**Table 2.** Calculated ingredient and nutrient content of the high or low standardized ileal digestible calcium diets or the reference diet, as-fed.

Ingredient, %	Standardized ileal digestible calcium, %		Reference diet
	0.60	0.20	
Wheat	10.00	10.00	10.00
Corn dried distillers grains with solubles	5.00	5.00	5.00
Corn	43.51	47.13	40.67
Soybean meal	31.12	30.52	32.93
Canola meal	3.50	3.50	3.50
Soya oil	2.19	1.04	3.40
Salt	0.32	0.32	0.32
Limestone	2.02	0.13	0.56
Dicalcium phosphate	1.06	1.06	2.33
Sodium bicarbonate	0.10	0.10	0.10
Lysine HCl	0.21	0.22	0.21
DL-Methionine	0.26	0.25	0.28
Threonine	0.07	0.07	0.09
Premix <sup>1</sup>	0.50	0.50	0.50
Protease <sup>2</sup>	0.02	0.02	0.00
Phytase <sup>3</sup>	0.03	0.03	0.00
Carbohydrase <sup>4</sup>	0.01	0.01	0.00
Titanium dioxide	0.10	0.10	0.10
Formulated nutrient content, %			
Crude protein	23.00	23.00	23.00
ME, kcal/kg	3,000.00	3,000.00	3,000.00
DM	87.72	87.34	87.92
Total calcium	1.22	0.50	0.96
SID calcium	0.60	0.20	0.42
Phosphorus	0.59	0.60	0.82
Available phosphorus	0.48	0.48	0.48
Non-phytate phosphorus	0.32	0.32	0.54
Phytate phosphorus	0.27	0.28	0.27
Digestible methionine + cystine	0.95	0.95	0.95
Digestible lysine	1.28	1.28	1.28
Digestible threonine	0.86	0.86	0.86
Sodium	0.18	0.18	0.18
Chloride	0.29	0.29	0.28

<sup>1</sup>Vitamin and mineral premix provided (per kilogram of starter diet): vitamin A 12,000 IU; vitamin D<sub>3</sub> 2,240 IU; 25-OH-D<sub>3</sub> 69 µg; vitamin E 150 IU; vitamin K<sub>3</sub> 3 mg; vitamin B<sub>1</sub> 3 mg; vitamin B<sub>2</sub> 8 mg; vitamin B<sub>6</sub> 4 mg; vitamin B<sub>12</sub> 0.02 mg; biotin 0.25 mg; folic acid 2 mg; niacinamide 60 mg; D-pantothenic acid 15 mg; Fe (as FeSO<sub>4</sub>) 40 mg; Cu (as CuCl<sub>2</sub>) 15 mg; Mn (as MnSO<sub>4</sub>) 110 mg; Zn (as ZnSO<sub>4</sub>) 90 mg; I (as KIO<sub>3</sub>) 0.5 mg; Se (as Na<sub>2</sub>SeO<sub>3</sub>) 0.25 mg; choline (as choline chloride) 400 mg.

<sup>2</sup>Ronozyme ProAct, CT (DSM Nutritional Products, Kaiseraugst, Switzerland) with an expected activity of 75,000 PROT/g, contributed 0.67% crude protein, 0.04% methionine + cysteine, 0.05% lysine, and 0.05% threonine.

<sup>3</sup>Ronozyme HiPhos GT (DSM Nutritional Products, Kaiseraugst, Switzerland) with an expected activity of 10,000 FYT/g, contributed 0.19% available P.

<sup>4</sup>Ronozyme MultiGrain (DSM Nutritional Products, Kaiseraugst, Switzerland) with an expected activity of 2,700 FXU/g and 700 FBG/g, contributed 65 kcal/kg metabolizable energy.

hydrolysates were determined by HPLC (Agilent Model 1260, Santa Clara, CA) coupled with pre-column derivatization with FITC. Amino acids, Ca, P, and Ti were then used to determine apparent ileal digestibility (**AID**), digestible nutrient intake and nutrient retention. Tibias were stripped of adhering tissues, dried and fat extracted prior to ashing for determination of tibia ash percent. Xylanase and protease activities in the SID Ca diets were analyzed using methods based on dye-labeled substrates (Azo-Xylan and Suc-Ala-Ala-Pro-Phe-pNA, respectively). Phytase activity was measured by Method PHY-102/06E DSM and one phytase unit was defined as the amount of enzyme that releases 1 µmol of inorganic phosphate from 50 mM phytate per minute at 37°C and pH 5.5.

### Statistical Analysis

Data were subjected to an analysis of variance using JMP Pro v. 15.0 (SAS Institute, Cary, NC). Cage served

as the experimental unit. Prior to statistical analyses, the distribution platform was used to verify normality. Any outliers, determined as 3 times the root mean square error plus or minus the mean of the response, were removed from the statistical analysis. Growth performance, livability, tibia ash, AID, and retention were analyzed using the fit model platform. Livability data were transformed using Box-Cox transformations in the fit model platform. For all parameters, the statistical model included diet and block. If diet effects were significant, means were separated using orthogonal contrast statements to determine linear and quadratic effects of SID Ca and Dunnett's Multiple Comparison tests to compare the reference diet (control group) against the diets formulated using SID Ca. Finally, the SID Ca requirement was estimated using the fit Y by X, fit special platform including linear or nonlinear models and uncentered polynomials in JMP Pro v. 15.0 (SAS, Cary, NC). Significance was accepted at  $P < 0.05$ .

## RESULTS AND DISCUSSION

The ingredient SID Ca coefficients used in the feed formulation to obtain the required dietary SID Ca concentrations are presented in [Table 1](#). Physiological properties of limestone, including particle size, in vitro solubility and geological location, can influence in vivo Ca digestibility ([Kim et al., 2019](#)). Therefore, the digestible Ca coefficient for limestone was estimated using the pH 3 glycine buffer equation for Ca digestibility established by [Kim et al. \(2019\)](#). This equation used in vitro solubility at 15 and 30 minutes and particle size and was a good predictor of in vivo Ca digestibility (adjusted  $R^2 = 0.98$ ; [Kim et al., 2019](#)). For all other ingredients the SID Ca coefficients were obtained from a review article by [Walk et al. \(2021\)](#). Due to the low contribution of Ca in endogenous losses in poultry, [Walk et al. \(2021\)](#) found no significant difference between the SID and AID Ca coefficients within the ingredients. Therefore, the mean of the digestible Ca coefficients for each ingredient was used to formulate the experimental diets ([Table 2](#)).

Formulated and analyzed total Ca and P and enzyme activity recovered in the experimental diets were as expected when considering analytical variation and overages in the enzyme premix ([Table 3](#)). Initial BW was  $49.9 \pm 0.2$  g and not influenced by treatment ( $P = 0.70$ ) or block ( $P = 0.77$ ). Broilers ate and gained approximately 17% less than Arbor Acres Plus breed guidelines, which may be the result of the housing system (battery cages vs. floor pens; [Santos et al., 2012](#)). Livability from hatch to d 10 post-hatch was 97.9% and not influenced by diet ( $P = 0.07$ ; [Table 4](#)). There was no linear or quadratic effect of SID Ca concentration on FI or BWG ([Table 4](#)). However, birds fed diets formulated to contain 0.60, 0.50, 0.30, or 0.20% SID Ca ate ( $P < 0.05$ ) or gained ( $P < 0.05$ ) more when compared with birds fed the reference diet. Birds fed 0.60% SID Ca were more efficient compared with birds fed 0.20% SID Ca (linear,  $P < 0.05$ ). There were no differences in FCR between birds fed diets formulated using SID Ca or birds fed the reference diet. The greater growth performance and intake of birds fed the SID Ca diets may be attributed to a few factors: the use of exogenous enzymes to

improve nutrient utilization ([Stefanello et al., 2015](#)), a high dose of phytase to result in nearly complete phytate destruction ([Walk and Olukosi, 2019](#); [Walk and Rama Rao, 2020](#)), and a more precise concentration of digestible Ca and P in the diet. The lack of any impact of graded concentrations of SID Ca on FI or gain was not expected and may be attributed to the inclusion of a high dose of dietary phytase ([Walk et al., 2012a](#)) in all of the SID Ca diets. The young age of the birds and a high Ca content in the residual yolk sac may be another factor contributing to the lack of an effect of SID Ca concentration on FI or gain in the current trial. For example, the Ca content of the yolk increases by 71% from embryonic d 0 to 17.5 ([Hopcroft et al., 2019](#)). Previous authors reported no difference in daily FI or daily gain of broilers fed 0.40, 0.60, or 0.80% total Ca from hatch to d 4 post-hatch, whereas further increases in total dietary Ca significantly decreased daily intake and gain ([Mansilla et al., 2020](#)).

Tibia ash percent was greatest in birds fed 0.50% SID Ca and decreased as the SID Ca content in the diet decreased to 0.20% (quadratic,  $P < 0.05$ ). A regression equation, using tibia ash percent, estimated the SID Ca requirement of Arbor Acres Plus broilers from hatch to d 10 post-hatch at 0.53% ([Figure 1](#)) and this corresponds to a digestible Ca to avP ratio of 1.10. These results are similar to those reported by [Angel \(2017\)](#) who recommended a digestible Ca to digestible P ratio of 1.15 in broilers from hatch to d 10 post-hatch.

To validate the use of the ingredient SID Ca coefficients and the estimated SID Ca requirement, it was important to compare the SID Ca diets with the nutrient adequate reference diet. In this regard, tibia ash percent was similar between birds fed the reference diet and birds fed diets formulated using SID Ca, except birds fed 0.20% SID Ca, which was lower ( $P < 0.05$ ) than tibia ash of birds fed the reference diet ([Table 5](#)). These results indicate the Ca and P content in the SID Ca diets was adequate for skeletal development, except at the lowest SID Ca concentration, and provide an extra level of confidence in the estimated SID Ca requirement predicted using tibia ash percent.

**Table 3.** Analyzed nutrient content and enzyme activities recovered in the experimental diets<sup>1</sup>.

Analyzed nutrients, %	Standardized ileal digestible calcium, %					Reference diet
	0.60	0.50	0.40	0.30	0.20	
Dry matter	90.6	90.3	90.5	90.4	90.3	90.3
Total calcium	1.18	1.01	0.80	0.64	0.50	0.89
Total phosphorus	0.62	0.62	0.60	0.60	0.61	0.80
Phytate phosphorus	0.34	0.32	0.34	0.33	0.34	0.32
Crude protein	23.1	23.0	22.8	22.4	22.2	23.6
Total lysine	1.36	1.33	1.33	1.32	1.30	1.40
Total methionine	0.40	0.39	0.38	0.35	0.38	0.37
Total cysteine	0.64	0.63	0.63	0.58	0.59	0.65
Total threonine	0.92	0.91	0.88	0.87	0.89	0.97
Enzyme activities recovered						
Protease, PROT/kg	13,267	13,865	14,837	15,754	17,194	0
Glucanase, FBG/kg	31	21	32	39	26	0
Xylanase, FXU/kg	338	266	379	372	305	50
Phytase, FYT/kg	3,315	3,149	4,040	3,603	3,418	167

<sup>1</sup>Diets were analyzed in duplicate.

**Table 4.** Growth performance of broilers fed graded levels of standardized ileal digestible calcium from hatch to 10 d of age<sup>1</sup>.

Standardized ileal digestible (SID) Ca, %	Feed intake, g	Body weight gain, g	mFCR <sup>2</sup> , g:g	Livability, %
0.60%	260*	239**	1.088	97.4
0.50%	262**	240**	1.091	98.4
0.40%	256	235	1.088	99.5
0.30%	263**	236	1.117	98.4
0.20%	264**	238*	1.109	95.3
Reference diet <sup>3</sup>	248	225	1.103	98.4
Standard error of the mean	3	3	0.006	0.98
<i>P</i> -value				
Diet <sup>4</sup>	0.0038	0.0127	0.0028	0.0739
Linear SID Ca	0.4174	0.5699	0.0019	-
Quadratic SID Ca	0.3545	0.4841	0.6155	-
Block	0.0208	0.0615	0.0084	0.5585

<sup>1</sup>Data are least square means of 12 birds per pen and 16 replicate pens per treatment.

<sup>2</sup>Mortality corrected feed conversion ratio.

<sup>3</sup>The reference diet was formulated to meet or exceed nutrient requirements for fast growing broilers, using 0.96% total calcium and 0.48% available P, without exogenous enzymes.

<sup>4</sup>If the effect of diet was significant ( $P < 0.05$ ), a Dunnett's Multiple Comparison test was performed to compare the least square means of birds fed the SID Ca diets against the least square means of birds fed the reference diet (control group).

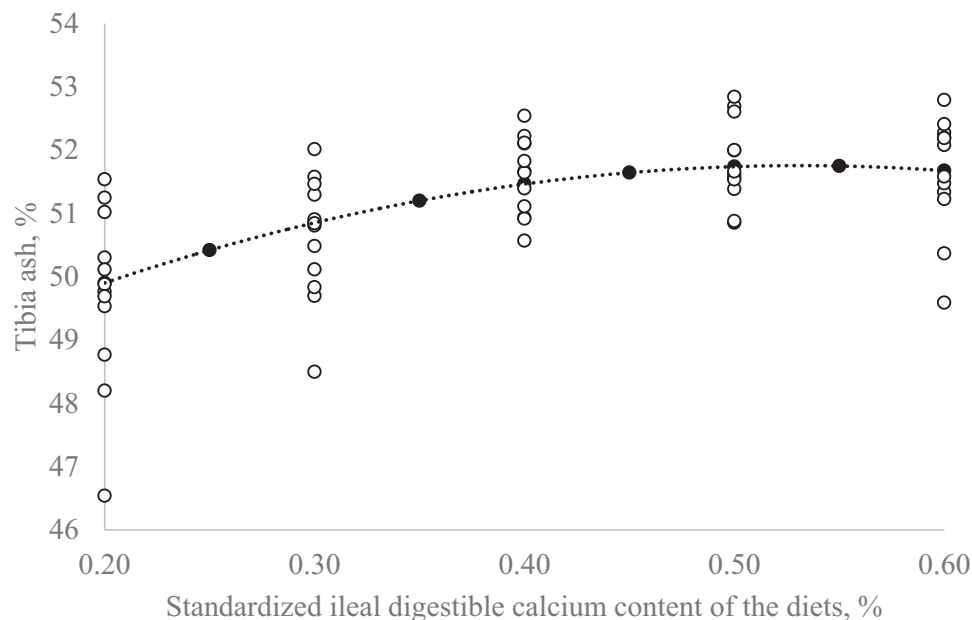
\* $P < 0.05$ .

\*\* $P < 0.01$ .

Apparent ileal digestibility or retention of P was greater ( $P < 0.05$ ) in birds fed all diets formulated using SID Ca when compared with birds fed the reference diet (Table 6). The inclusion of high doses of dietary phytase in the SID Ca diets would have contributed to the increase in P digestibility and retention compared with birds fed the reference diet, which contained no exogenous enzymes. However, reducing dietary Ca has also been reported to significantly improve P digestibility (Walk et al., 2012b; Amerah et al., 2014) and in the current study, the AID of P was improved as the SID Ca content of the diet decreased from 0.60 to 0.20% (linear,  $P < 0.05$ ). Decreasing the dietary SID Ca from 0.60 to 0.20% also increased (linear,  $P < 0.05$ ) digestible P

intake. Digestible P intake was lower ( $P < 0.05$ ) in birds fed diets formulated to contain 0.60, 0.50, or 0.40% SID Ca and greater ( $P < 0.05$ ) in birds fed 0.20% SID Ca had compared with birds fed the reference diet (Table 6). Finally, P retention was greatest in birds fed 0.50% SID Ca and decreased as the SID of Ca in the diet decreased to 0.20% (quadratic,  $P < 0.05$ ; Table 6). A regression equation, using P retention, estimated 100% of the SID Ca requirement of Arbor Acres Plus broilers from hatch to d 10 post-hatch at 0.49% (Figure 2), which corresponds to a digestible Ca to available P ratio in the diet of 1.02.

The AID of Ca was greatest ( $P < 0.05$ ) in birds fed 0.40, 0.30, or 0.20% SID Ca compared with birds fed the



**Figure 1.** Predicted (●) and measured (○) tibia ash percent of 10-day-old broilers fed graded concentrations of standardized ileal digestible (SID) Ca. Regression equation [ $= 46.992 + 17.937 \times \text{SID Ca, \%} - 16.882 \times \text{SID Ca, \%}^2$ ,  $R^2 = 0.40$ ,  $\text{RMSE} = 0.84$ ,  $P = 0.0129$ ] estimated 100% of the SID Ca requirement of Arbor Acre Plus broilers from hatch to 10 d post-hatch at 0.53% and this corresponds to a digestible Ca to available P ratio in the diet of 1.10.

**Table 5.** Tibia ash percent of broilers fed graded levels of standardized ileal digestible calcium from hatch to 10 d of age<sup>1</sup>.

Standardized ileal digestible (SID) Ca, %	Tibia ash, %
0.60%	51.63
0.50%	51.80
0.40%	51.59
0.30%	50.63
0.20%	49.98*
Reference diet <sup>2</sup>	51.42
Standard error of the mean	0.23
<i>P</i> -value	
Diet <sup>3</sup>	< 0.0001
Linear SID Ca	< 0.0001
Quadratic SID Ca	0.0080
Block	0.1413

<sup>1</sup>Data are least square means of 2 birds of average body weight per pen, pooled, and 12 replicate pens per treatment.

<sup>2</sup>The reference diet was formulated to meet or exceed nutrient requirements for fast growing broilers, using 0.96% total calcium and 0.48% available P, without exogenous enzymes.

<sup>3</sup>If the effect of diet was significant ( $P < 0.05$ ), a Dunnett's Multiple Comparison test was performed to compare the least square means of birds fed the SID Ca diets against the least square means of birds fed the reference diet (control group).

\* $P < 0.01$ .

reference diet (Table 6). Calcium retention was lower ( $P < 0.05$ ) in birds fed 0.60% SID Ca and greater ( $P < 0.05$ ) in birds fed 0.40, 0.30, or 0.20% SID Ca compared with birds fed the reference diet (Table 6). The AID or retention of Ca was similar between birds fed 0.60 or 0.50% SID Ca and increased as SID Ca in the diet decreased to 0.20% (quadratic,  $P < 0.05$ ). Birds fed 0.20% SID Ca had a lower digestible Ca intake compared with birds fed 0.60% SID Ca (linear,  $P < 0.05$ ; Table 6). The digestible intake of Ca was greater ( $P < 0.05$ ) in birds fed 0.60 or 0.50% SID Ca and lower ( $P < 0.05$ ) in birds fed 0.30 or 0.20% SID Ca compared with birds fed the reference diet. Previous authors have reported no effect of decreasing total dietary Ca on Ca digestibility (Mutucumarana et al., 2014) while others have reported

significant increases in Ca digestibility (Gautier et al., 2017) as total Ca decreased in the diet.

The negative impact of increasing dietary Ca (particularly from limestone) on amino acid digestibility has been previously reported (Amerah et al., 2014). Formulating diets on a SID Ca basis may improve the AID of amino acids by removing the detrimental impact of excess or nondigested dietary Ca. In the current study, the AID of only His, Pro, Leu, Met, or Met + Cys were influenced by the SID Ca content in the diet (Table 7). In general, the AID of His, Met or Met + Cys linearly ( $P < 0.05$ ) decreased as the SID Ca content of the diet decreased (Table 7), whereas birds fed 0.50% SID Ca had the greatest AID of Pro or Leu and digestibility decreased as the SID Ca concentration of the diet decreased to 0.20% (quadratic,  $P < 0.05$ ). These results are contradictory to those of Amerah et al. (2014) who reported an improvement in the AID of amino acids as total dietary Ca decreased from 1.30 to 0.51% and may highlight differences associated with formulating diets using SID Ca instead of total Ca.

In the current experiment, there was no difference in the AID of amino acids, except the AID of Pro, Met, or Met + Cys when birds were fed the reference diet compared with birds fed diets formulated using all levels of SID Ca (Table 7). However, FI was greater in birds fed diets formulated using SID Ca coefficients and this resulted in a greater ( $P < 0.05$ ) digestible amino acid intake of Asp, His, Pro, Phe, Met, or Met + Cys compared with birds fed the reference diet (Table 8). This was especially true at the higher concentrations of dietary SID Ca and the digestible intake of Glu, Ser, Gly, Pro, Cys, or Met + Cys decreased as the concentration of SID Ca in the diet decreased from 0.60 to 0.20% (linear,  $P < 0.05$ ; Table 8). Whereas for His, Phe, or Met, digestible intake was greatest in birds fed intermediate concentrations of SID Ca, resulting in a quadratic ( $P <$

**Table 6.** Apparent ileal Ca and P digestibility (AID), apparent digestible Ca and P intake, or retained Ca and P of broilers fed graded levels of standardized ileal digestible calcium from hatch to 10 d of age<sup>1</sup>.

Standardized ileal digestible (SID) Ca, %	AID Ca, %	AID P, %	Digestible Ca intake, g/b/d	Digestible P intake, g/b/d	Ca retention, %	P retention, %
0.60%	51.4	59.7*	0.157***	0.095***	43.2***	61.4***
0.50%	53.3	64.7***	0.139*	0.104***	47.8	62.0***
0.40%	59.3***	73.0***	0.121	0.112*	55.2***	61.8***
0.30%	69.1***	79.5***	0.117*	0.126	63.6***	59.6***
0.20%	77.1***	84.9***	0.102***	0.137**	75.7***	55.9***
Reference diet <sup>2</sup>	53.9	56.2	0.128	0.122	48.2	43.3
Standard error of the mean	0.70	0.91	0.003	0.002	0.62	0.49
<i>P</i> -value						
Diet <sup>3</sup>	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Linear SID Ca	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Quadratic SID Ca	< 0.0001	0.7375	0.0698	0.2803	< 0.0001	< 0.0001
Block	0.5809	0.1076	0.2364	0.0738	0.0131	0.0267

<sup>1</sup>Data are least square means from 12 birds per pen, pooled, and 12 replicate pens per treatment.

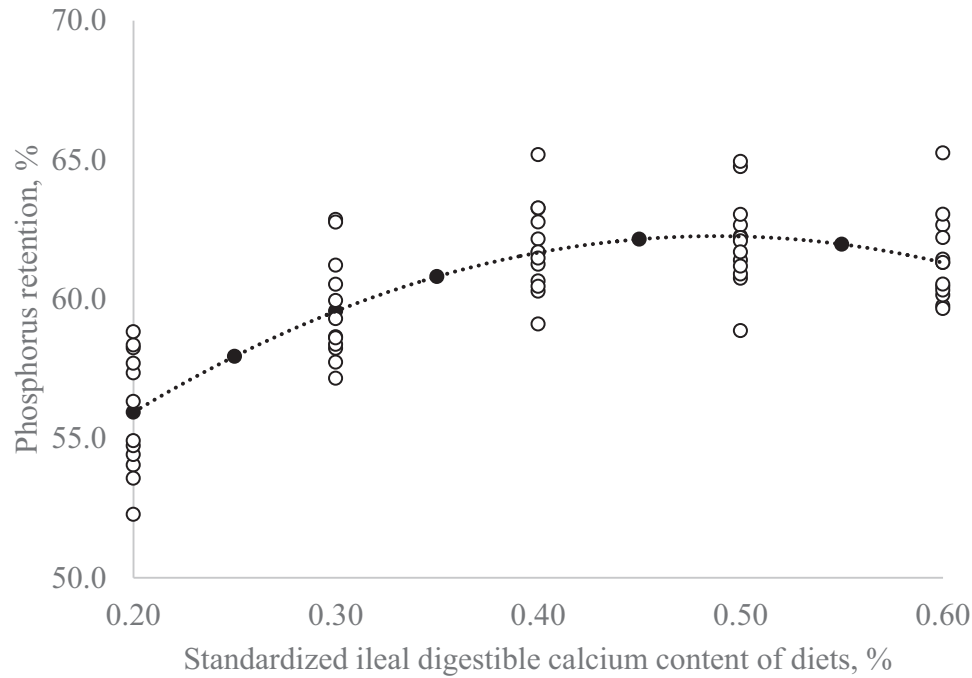
<sup>2</sup>The reference diet was formulated to meet or exceed nutrient requirements for fast growing broilers, using 0.96% total calcium and 0.48% available P, without exogenous enzymes.

<sup>3</sup>If the effect of diet was significant ( $P < 0.05$ ), a Dunnett's Multiple Comparison test was performed to compare the least square means of birds fed the SID Ca diets against the least square means of birds fed the reference diet (control group).

\* $P < 0.05$ .

\*\* $P < 0.01$ .

\*\*\* $P < 0.0001$ .



**Figure 2.** Predicted (●) and measured (○) phosphorus retention of 10-day-old broilers fed graded concentrations of standardized ileal digestible (SID) Ca. Regression equation [ $= 44.119 + 74.345 \times \text{SID Ca, \%} - 76.149 \times \text{SID Ca, \%}^2$ ,  $R^2 = 0.63$ ,  $\text{RMSE} = 1.79$ ,  $P < 0.0001$ ] estimated 100% of the SID Ca requirement of Arbor Acre Plus broilers from hatch to d 10 post-hatch is 0.49% and this corresponds to a digestible Ca to available P ratio in the diet of 1.02.

0.05) influence of dietary SID Ca on digestible amino acid intake (Table 8).

Interest in using digestible requirements of nutrients, such as amino acids and P, promotes the prudent use of feed ingredients, reduces nutrient excretion into the

environment, may reduce feed costs, as well as optimizes interactions or ratios with other nutrients in the diet. Formulating broiler diets using digestible amino acids significantly improved growth performance, feed efficiency and profitability when compared with the use of

**Table 7.** Apparent ileal amino acid digestibility (AID) of broilers fed graded levels of standardized ileal digestible calcium from hatch to 10 d of age<sup>1</sup>.

AID, %	Standardized ileal digestible calcium, %					Reference diet <sup>2</sup>	SEM	Diet <sup>3</sup>	P-value		
	0.60	0.50	0.40	0.30	0.20				SID Ca		
									Linear	Quadratic	Block
Asp	76.9	78.1	78.5	76.8	76.9	74.8	0.84	0.0628	-	-	0.0091
Glu	84.4	85.3	84.8	83.6	82.7	83.7	0.66	0.0810	-	-	0.1630
Ser	76.0	76.9	75.9	73.9	73.5	74.5	0.96	0.1009	-	-	0.1009
Gly	73.4	73.4	73.8	72.0	70.1	71.3	1.17	0.1875	-	-	0.0331
His	81.5	81.6	79.0	78.4	77.2	79.3	0.88	0.0038	0.0001	0.8450	< 0.0001
Arg	84.5	85.1	84.8	83.9	84.0	83.8	0.79	0.7956	-	-	0.0060
Thr	72.2	72.7	71.6	69.6	69.6	71.3	1.18	0.3129	-	-	0.2062
Ala	76.7	77.9	77.6	75.5	74.8	75.7	0.99	0.1433	-	-	0.0233
Pro	78.9	79.7*	78.4	76.3	73.9	76.6	0.73	< 0.0001	< 0.0001	0.0103	0.0728
Tyr	76.1	77.4	77.3	74.5	75.0	74.4	1.00	0.1208	-	-	0.0289
Val	76.7	77.7	78.3	76.2	75.3	76.0	0.93	0.2175	-	-	0.0013
Ile	76.7	78.0	78.5	76.6	75.7	75.5	0.86	0.1056	-	-	0.0020
Leu	78.9	80.0	79.7	77.7	76.3	77.2	0.83	0.0156	0.0096	0.0369	0.0089
Phe	77.9	79.7	80.7	78.8	78.1	77.3	0.83	0.0507	-	-	0.0213
Lys	79.8	81.2	81.2	79.1	79.1	79.5	0.94	0.4093	-	-	0.0016
Met	74.3**	74.7**	70.8	65.2	66.1	67.5	1.39	< 0.0001	< 0.0001	0.8769	0.0218
Cys	89.9	90.6	89.8	88.1	88.4	88.5	0.71	0.0951	-	-	0.0490
Trp	69.2	71.9	70.4	67.6	68.1	72.4	1.65	0.2322	-	-	0.0008
Met + Cys	83.9	84.5*	82.6	79.5	79.7	80.9	0.94	0.0005	< 0.0001	0.5412	0.0394
Total AA	79.4	80.3	79.9	78.1	77.4	77.6	0.86	0.0898	-	-	0.0537

<sup>1</sup>Data are least square means from 12 birds per pen, pooled, and 12 replicate pens per treatment.

<sup>2</sup>The reference diet was formulated meet or exceed nutrient requirements for fast growing broilers, using 0.96% total calcium and 0.48% available P in the feed formulation, without exogenous enzymes.

<sup>3</sup>If the effect of diet was significant ( $P < 0.05$ ), a Dunnett's Multiple Comparison test was performed to compare the least square means of birds fed the SID Ca diets against the least square means of birds fed the reference diet (control group).

\* $P < 0.05$ .

\*\* $P < 0.01$ .

**Table 8.** Apparent digestible amino acid intake (g/d) of broilers fed graded levels of standardized ileal digestible calcium from hatch to 10 days of age<sup>1</sup>.

Digestible intake, g/d	Standardized ileal digestible calcium, %					Reference diet <sup>2</sup>	SEM	P-value			
	0.60	0.50	0.40	0.30	0.20			Diet <sup>3</sup>	Linear	Quadratic	Block
Asp	0.39	0.40**	0.41**	0.40*	0.40**	0.37	0.007	0.0050	0.2248	0.3083	0.0005
Glu	1.01	1.03	0.97	0.97	0.96	0.98	0.016	0.0124	0.0018	0.9101	0.0082
Ser	0.22	0.23	0.22	0.21	0.21	0.21	0.004	0.0381	0.0075	0.9914	0.0265
Gly	0.19	0.19	0.18	0.18	0.17	0.18	0.004	0.0071	0.0004	0.4670	0.0022
His	0.15***	0.14**	0.13	0.13	0.13	0.13	0.002	< 0.0001	< 0.0001	0.0036	< 0.0001
Arg	0.32	0.32	0.31	0.31	0.31	0.31	0.005	0.7734	-	-	0.0003
Thr	0.17	0.17	0.16	0.16	0.16	0.17	0.003	0.0550	-	-	0.0095
Ala	0.23	0.23	0.23	0.23	0.22	0.23	0.004	0.3787	-	-	0.0007
Pro	0.32**	0.33***	0.31	0.31	0.29	0.30	0.005	< 0.0001	0.0001	0.0731	0.0023
Tyr	0.13	0.13	0.13	0.12	0.13	0.13	0.002	0.1801	-	-	0.0004
Val	0.21	0.21	0.22	0.21	0.21	0.21	0.004	0.2816	-	-	0.0002
Ile	0.20	0.21	0.21	0.21	0.20	0.20	0.004	0.1360	-	-	0.0004
Leu	0.42	0.43	0.43	0.42	0.41	0.41	0.007	0.0961	-	-	0.0004
Phe	0.20	0.21	0.22**	0.22	0.21	0.21	0.004	0.0012	0.0073	0.0016	0.0014
Lys	0.28	0.28	0.27	0.27	0.27	0.28	0.005	0.8445	-	-	0.0008
Met	0.08***	0.08***	0.07**	0.06	0.07	0.06	0.002	< 0.0001	< 0.0001	0.0359	0.0002
Cys	0.15	0.15	0.14	0.13	0.14	0.14	0.002	0.0002	< 0.0001	0.9134	0.0017
Trp	0.04*	0.04	0.04*	0.04*	0.04*	0.05	0.001	0.0286	0.8447	0.8009	0.0036
Met+Cys	0.23**	0.22**	0.21	0.19	0.20	0.20	0.004	< 0.0001	< 0.0001	0.3156	0.0005
Total AA	4.73	4.77	4.66	4.61	4.53	4.54	0.080	0.1910	-	-	0.0014

<sup>1</sup>Data are least square means from 12 birds per pen, pooled, and 12 replicate pens per treatment.

<sup>2</sup>The reference diet was formulated meet or exceed nutrient requirements for fast growing broilers, using 0.96% total calcium and 0.48% available P in the feed formulation, without exogenous enzymes.

<sup>3</sup>If the effect of diet was significant ( $P < 0.05$ ), a Dunnett's Multiple Comparison test was performed to compare the least square means of birds fed the SID Ca diets against the least square means of birds fed the reference diet (control group).

\* $P < 0.05$

\*\* $P < 0.01$

\*\*\* $P < 0.0001$ .

total amino acids in feed formulations (Rostagno et al., 1995; Dari et al., 2005). In the current experiment, while the benefits of the exogenous enzymes in the SID Ca diets cannot be ruled out, the formulation of diets using digestible Ca coefficients compared with total Ca concentrations contributed to greater growth performance and improvements in the AID or retention of P, Ca and a limited number of amino acids when compared with birds fed the reference diet.

Regression equations, developed using tibia ash, Ca or P digestibility, digestible intake, and retention are presented in Table 9. Response variables that are the most sensitive to Ca and P concentrations in the diet are bone ash, growth performance, and Ca and P digestibility. Growth rate and efficiency are optimized at concentrations of Ca that are suboptimal for bone mineralization

(Bedford and Rousseau, 2017; Lagos et al., 2019; Mansilla et al., 2020). In the current experiment, there was no effect of graded levels of SID Ca on body weight gain and the linear impact of dietary SID Ca on FCR or the AID of P or Ca suggest the requirement is greater than or less than that tested in the current experiment for these parameters. However, there was a nonlinear effect of SID Ca on bone ash percent and apparent P retention. These response variables estimated the SID Ca requirement for Arbor Acres Plus broilers from hatch to 10 d post-hatch, when fed diets nearly devoid of dietary phytate, was 0.53 and 0.49%, respectively (Table 9). This corresponds to an SID Ca to available P ratio of between 1.10 and 1.02. Furthermore, there was a good relationship ( $R^2 = 0.92$ ,  $P < 0.001$ ; Figure 3) between the formulated SID Ca concentrations and the measured

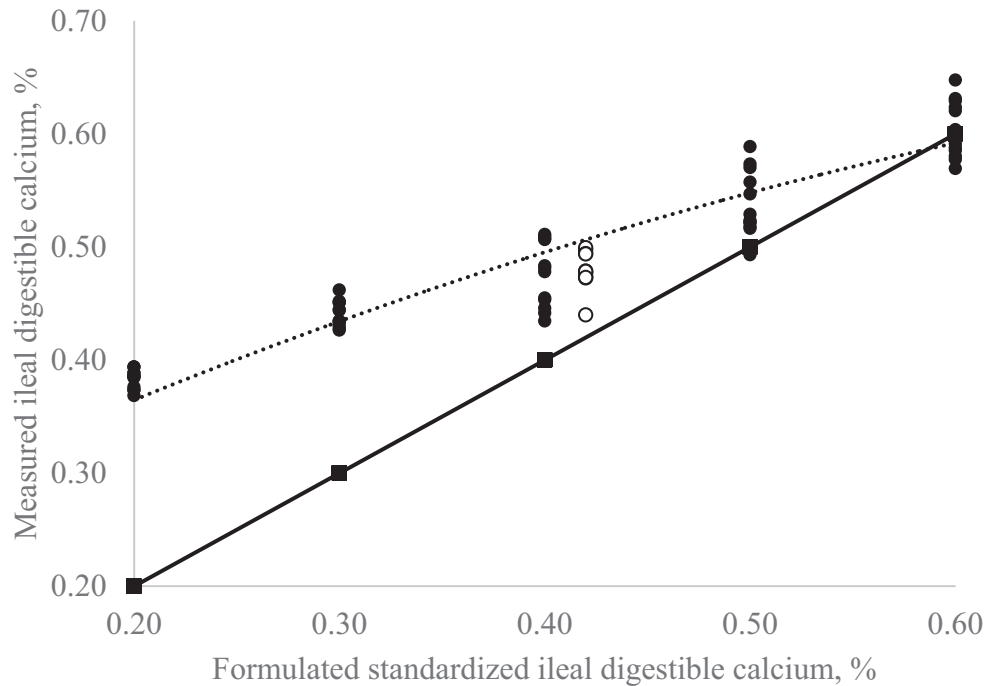
**Table 9.** Prediction models for broiler growth performance, tibia ash, apparent ileal Ca or P digestibility, and apparent Ca or P retention when fed graded concentrations of standardized ileal digestible (SID) calcium from hatch to d 10 post-hatch<sup>1</sup>.

Response variable	Model	R <sup>2</sup>	RMSE	P-value	Estimated SID Ca requirement, %
Tibia ash, %	$= 46.992 + 17.937 \times \text{SID Ca, \%} - 16.882 \times \text{SID Ca, \%}^2$	0.40	0.84	0.0129	0.53
AID <sup>2</sup> Ca, %	$= 104.951 - 158.527 \times \text{SID Ca, \%} + 113.935 \times \text{SID Ca, \%}^2$	0.93	2.63	< 0.0001	< 0.20
Digestible Ca intake, g/b/d	$= 0.074 + 0.133 \times \text{SID Ca, \%}$	0.79	0.01	< 0.0001	> 0.60
AID P, %	$= 98.407 - 65.113 \times \text{SID Ca, \%}$	0.88	3.49	< 0.0001	< 0.20
Digestible P intake, g/b/d	$= 0.157 - 0.104 \times \text{SID Ca, \%}$	0.73	0.01	< 0.0001	< 0.20
Apparent Ca retention, %	$= 105.302 - 171.558 \times \text{SID Ca, \%} + 113.411 \times \text{SID Ca, \%}^2$	0.96	2.41	< 0.0001	< 0.20
Apparent P retention, %	$= 44.119 + 74.345 \times \text{SID Ca, \%} - 76.149 \times \text{SID Ca, \%}^2$	0.63	1.79	< 0.0001	0.49

<sup>1</sup>Only response variables that were significantly ( $P < 0.05$ ) influenced by the SID Ca content in the diet were used to estimate the SID Ca requirement of broilers from hatch to d 10 post-hatch.

<sup>2</sup>Apparent ileal digestible.





**Figure 3.** Relationship between the formulated ( $\square$ ) and measured digestible ( $\bullet$ ) ileal Ca of 10-day-old broilers fed graded concentrations of standardized ileal digestible (SID) Ca. The measured digestible Ca was 0.60, 0.54, 0.48, 0.44, or 0.38% for broilers fed diets formulated to contain 0.60, 0.50, 0.40, 0.30, or 0.20% SID Ca, respectively. Adjusted  $R^2 = 0.92$ , RMSE = 0.02,  $P < 0.0001$ . The analyzed digested ileal Ca of birds fed the PC ( $\circ$ ) was 0.48% compared with the formulated SID Ca of 0.42% or formulated total Ca of 0.96%.

Ca digested by the birds, particularly in birds fed 0.60 or 0.50% SID Ca, and this corresponded to a measured digestible Ca of 0.60 or 0.54%, respectively. However, as the SID Ca content in the diet decreased below the estimated requirements, the AID of Ca increased and this resulted in an 8, 14, or 18 percentage unit increase in the measured Ca digested by birds fed 0.40, 0.30, or 0.20% SID Ca, respectively. Birds will regulate Ca and P homeostasis via the kidneys, bones, plasma, and small intestine (Proszkowiec-Weglarz and Angel, 2013). The extent of how Ca and P are regulated is dependent on the ratio and sufficiency of either nutrient in the diet. In a P-adequate diet, as in the current trial, birds fed SID Ca below the estimated requirement increased Ca digestibility in the small intestine which resulted in a deviation from the expected values. The lack of a need to upregulate Ca utilization (i.e., no increase digestibility) in birds fed diets containing 0.60 or 0.50% SID Ca further support the estimated SID Ca requirement is between 0.53 and 0.49% for fast-growing broilers from hatch to d 10 post-hatch.

In conclusion, formulating broiler diets using SID Ca coefficients resulted in significant improvements in body weight gain, FCR, and P and Ca digestibility or retention compared with broilers fed diets formulated using total Ca and without enzyme supplementation. These results indicate further improvements in nutrient utilization and efficiency are possible with more precise feed formulation applications, such as using SID Ca coefficients for feed ingredients. Bone ash and P retention data suggest the SID Ca requirement of male, Arbor Acres Plus broilers from hatch to d 10 post-hatch is between 0.49 and 0.53%. This corresponds to a SID Ca

to available P ratio between 1.02 and 1.10. Confirmation of these results, the impact of available or SID P concentration, and determination of the SID Ca requirements of older birds and of different genetic strains is required to implement SID Ca requirements as standard practice in broiler feed formulations. These initial results suggest formulating broiler diets using SID Ca coefficients and phytase to nearly completely remove phytate, results in benefits beyond feed efficiency and bone ash, such as improved amino acid utilization and P retention.

## DISCLOSURES

The authors declare no conflict of interest.

## REFERENCES

- Amerah, A. M., P. W. Plumstead, L. P. Barnard, and A. Kumar. 2014. Effect of calcium level and phytase addition on ileal phytate degradation and amino acid digestibility of broilers fed corn-based diets. *Poult. Sci.* 93:906–915.
- Angel, C. R. 2017. Rethinking calcium and phosphorus nutrition in poultry: the importance of calcium digestibility. XXXIII Curso De Especializacion FEDNA. *Adv. Nutr. Aliment. Anim.* 141–147 Madrid.
- Anwar, M. N., V. Ravindran, P. C. H. Morel, G. Ravindran, and A. J. Cowieson. 2015. Measurement of true ileal calcium digestibility in meat and bone meal for broiler chickens. *An. Feed Sci. Tech.* 206:100–107.
- Anwar, M. N., V. Ravindran, P. C. H. Morel, G. Ravindran, and A. J. Cowieson. 2016a. Measurement of true ileal calcium digestibility in meat and bone meal for broiler chickens using the direct method. *Poult. Sci.* 95:70–76.
- Anwar, M. N., V. Ravindran, P. C. H. Morel, G. Ravindran, and A. J. Cowieson. 2016b. Apparent ileal digestibility of calcium in limestone for broiler chickens. *An. Feed Sci. Tech.* 213:142–147.

- Anwar, M. N., V. Ravindran, P. C. Morel, G. Ravindran, and A. J. Cowieson. 2016c. Effect of limestone particle size and calcium to non-phytate phosphorus ratio on true ileal calcium digestibility of limestone for broiler chickens. *Br. Poult. Sci.* 57:707–713.
- Anwar, M. N., V. Ravindran, P. C. H. Morel, G. Ravindran, and A. J. Cowieson. 2017. Effect of calcium source and particle size on the true ileal digestibility and total tract retention of calcium in broiler chickens. *An. Feed Sci. Tech.* 224:39–45.
- Anwar, M. N., V. Ravindran, P. C. H. Morel, G. Ravindran, and A. J. Cowieson. 2018. Measurement of the true ileal calcium digestibility of some feed ingredients for broiler chickens. *An. Feed Sci. Tech* 237:118–128.
- AOAC. 2006. *Official Methods of Analysis of AOAC International* (18th ed.). AOAC, Arlington, VA.
- Bedford, M., and X. Rousseau. 2017. Recent findings regarding calcium and phytase in poultry nutrition. *Anim. Prod. Sci.* 57:2311–2316.
- Cowieson, A. J., F. F. Roos, J.-P. Ruckebusch, J. W. Wilson, P. Guggenbuhl, H. Lu, K. M. Ajuwon, and O. Adeola. 2017. Time-series responses of swine plasma metabolites to ingestion of diets containing myo-inositol or phytase. *Br. J. Nutr.* 118:897–905.
- Dari, R. L., A. M. Penz Jr., A. M. Kessler, and H. C. Jost. 2005. Use of digestible amino acids and the concept of ideal protein in feed formulation for broilers. *J. Appl. Poult. Res.* 14:195–203.
- David, L. S., M. R. Abdollahi, G. Ravindran, C. L. Walk, and V. Ravindran. 2019. Studies on the measurement of ileal calcium digestibility of calcium sources in broiler chickens. *Poult. Sci.* 98:5582–5589.
- Gautier, A. E., C. L. Walk, and R. N. Dilger. 2017. Influence of dietary calcium concentrations on the calcium to non-phytate phosphorus ratio on growth performance, bone characteristics, and digestibility in broilers. *Poult. Sci.* 96:2795–2803.
- Hopcroft, R. L., A. J. Cowieson, W. I. Muir, and P. J. Groves. 2019. Changes to mineral levels in the yolk of meat chicken embryos during incubation. *Poult. Sci.* 98:1511–1516.
- Kim, S. W., W. Li, R. Angel, and P. W. Plumstead. 2019. Modification of a limestone solubility method and potential to correlate with in vivo limestone calcium digestibility. *Poult. Sci.* 98:6837–6848.
- Kim, S. W., W. Li, R. Angel, and M. Proszkowiec-Weglarz. 2018. Effects of limestone particle size and dietary Ca concentration on apparent P and Ca digestibility in the presence or absence of phytase. *Poult. Sci.* 97:4306–4314.
- Lagos, L. V., C. L. Walk, M. R. Murphy, and H. H. Stein. 2019. Effects of dietary digestible calcium on growth performance and bone ash concentration in 50- to 85-kg growing pigs fed diets with different concentrations of digestible phosphorus. *Anim. Feed Sci. Technol.* 247:262–272.
- Mansilla, W. D., R. Franco-Rossello, C. A. Torres, A. Dijkslag, and A. I. Garcia-Ruiz. 2020. The effect of reducing dietary calcium in pre-starter diets (0-4 D) on growth performance of broiler chickens, tibia characteristics, and calcium and phosphorus concentration in the blood. *Poult. Sci.* 99:4904–4913.
- Mutucumarana, R. K., V. Ravindran, G. Ravindran, and A. J. Cowieson. 2014. Influence of dietary calcium concentration on the digestion of nutrients along the intestinal tract of broiler chickens. *J. Poult. Sci.* 51:392–401.
- Paiva, D. M., C. L. Walk, and A. P. McElroy. 2013. Influence of dietary calcium level, calcium source, and phytase on bird performance and mineral digestibility during a natural necrotic enteritis episode. *Poult. Sci.* 92:3125–3133.
- Paiva, D. M., C. L. Walk, and A. P. McElroy. 2014. Dietary calcium, phosphorus, and phytase effects on bird performance, intestinal morphology, mineral digestibility and bone ash during a natural necrotic enteritis episode. *Poult. Sci.* 93:2753–2762.
- Proszkowiec-Weglarz, M., and R. Angel. 2013. Calcium and phosphorus metabolism in broilers: effect of homeostatic mechanism on calcium and phosphorus digestibility. *J. Appl. Poult. Res.* 22:609–627.
- Rostagno, H. S., J. M. R. Pupa, and M. Pack. 1995. Diet formulation for broilers based on total versus digestible amino acids. *J. Appl. Poult. Res.* 4:293–299.
- Santos, F. B. O., A. A. Santos Jr., E. O. Oviedo-Rondon, and P. R. Ferket. 2012. Influence of housing system on growth performance and intestinal health of Salmonella-challenged broiler chickens. *Curr. Res. Poult. Sci.* 2:1–10.
- Stefanello, C., S. L. Vieira, G. O. Santiago, L. Kindlein, J. O. B. Sorbara, and A. J. Cowieson. 2015. Starch digestibility, energy utilization, and growth performance of broilers fed corn-soybean meal basal diets supplemented with enzymes. *Poult. Sci.* 94:2472–2479.
- Walk, C. L., L. F. Romero, and A. J. Cowieson. 2021. Towards a digestible calcium system for broiler chicken nutrition: a review and recommendations for the future. *Anim. Feed Sci. Technol.* 276:114930.
- Walk, C. L., and S. V. Rama Rao. 2020. Increasing dietary phytase has a significant anti-nutrient effect on apparent ileal amino acid digestibility and digestible amino acid intake requiring increasing doses of phytase as evidenced by prediction equations. *Poult. Sci.* 99:290–300.
- Walk, C. L., and O. A. Olukosi. 2019. Influence of graded concentrations of phytase in high-phytate diets on growth performance, apparent ileal amino acid digestibility, and phytate concentration in broilers from hatch to day 28 post-hatch. *Poult. Sci.* 98:3884–3893.
- Walk, C. L., E. K. Addo-Chidie, M. R. Bedford, and O. Adeola. 2012a. Evaluation of a highly soluble calcium source and phytase in the diets of broiler chickens. *Poult. Sci.* 91:2255–2263.
- Walk, C. L., M. R. Bedford, and A. P. McElroy. 2012b. Influence of limestone and phytase on broiler performance, gastrointestinal pH, and apparent ileal nutrient digestibility. *Poult. Sci.* 91:1371–1378.
- Zhang, F., and O. Adeola. 2018. True ileal digestibility of calcium in limestone and dicalcium phosphate are additive in diets of broiler chickens. *Poult. Sci.* 97:4290–4296.