Determination of the standardized ileal digestible calcium requirement of male Arbor Acres Plus broilers from day 11 to 24 post-hatch

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ABSTRACT Male broilers (n = 576) were obtained and allocated to 96 cages with 6 birds per cage. From hatch to d 24, 16 randomly allocated cages were fed a nutrient adequate reference diet. The reference diet was formulated to contain 0.96 and 0.87% total Ca from hatch to d 10 and d 11 to 24, respectively. The remaining 80 cages were fed a nutrient adequate diet, formulated to contain 0.50% standardized ileal digestible (**SID**) Ca from hatch to d 10. On d 11, five diets containing 0.56, 0.46, 0.36, 0.26, or 0.16% SID Ca were randomly assigned to 16 cages per diet. Means were separated using polynomial contrasts and Dunnett's Multiple Comparison tests. From hatch to d 10, birds fed diets formulated to contain 0.50% SID Ca gained more ($P < 0.05$) compared with birds fed the reference diet. From d 11 to 24, there was no effect of diet on feed intake or BW gain. Birds fed 0.46 or 0.16% SID Ca were less ($P < 0.05$) efficient compared with birds fed the reference diet. Birds fed 0.26 or 0.36% SID Ca were more efficient compared with birds fed all other levels of SID Ca (quadratic, $P < 0.05$). Tibia ash percent was greatest in birds fed 0.56 or 0.46% SID Ca, decreased (quadratic, $P < 0.05$) as dietary SID Ca decreased and was lower ($P < 0.001$) in birds fed the 0.26 or 0.16% SID Ca diets compared with birds fed the reference diet. Apparent ileal digestibility (**AID**) of Ca or retention of P was greater ($P < 0.05$) in birds fed the diets formulated using SID Ca compared with birds fed the reference diet. Apparent P retention was greatest in birds fed 0.36% SID Ca (quadratic, $P < 0.05$). Regression equations developed using P retention, AID of Ca, and percent tibia ash estimated the SID Ca requirement of 11 to 24-d-old broilers was 0.380, 0.488, and 0.515%, respectively. This corresponds to a SID Ca to available P ratio of 0.86 to 1.17.

Key words: broiler, digestible calcium, phosphorus, tibia ash

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INTRODUCTION

Accurate formulation of dietary Ca and P is important for optimal broiler growth, skeletal development, and nutrient utilization. Imbalances in dietary Ca or P will negatively influence growth rate, skeletal development, and nutrient digestibility in both broilers (Amerah et al., 2014) and pigs (Lagos et al., 2019). Broiler diets are currently formulated to meet P requirements using digestible, non-phytate or available P (**avP**) formulation systems. Regardless of the formulation system employed for P, there appears to be a good agreement ($R^2 = 0.75$) between the total P formulated and analyzed in the diet (Walk, 2016). In contrast, dietary Ca is currently formulated using total Ca and excess or large variation in the formulated and analyzed concentration of Ca in the diets is often reported ($R^2 = 0.37$; Walk, 2016). This excess or 'unaccounted for' total dietary Ca may be contributing to poor growth rates and skeletal development and reduced nutrient utilization in broiler chickens.

Recently, considerable efforts have been made to determine apparent, standardized, or true ileal Ca digestibility coefficients of ingredients for broilers (Anwar et al., 2015,2016a,b,c, 2017, 2018; Zhang and Adeola, 2018; David et al., 2019; David et al., 2020). Walk et al. (2021a) presented the mean, minimum and maximum apparent or standardized ileal digestible (**SID**) Ca coefficients for a few commonly used feed ingredients and found no significant difference in the Ca digestibility coefficients if apparent or standardized values were employed (Walk et al., 2021a). Therefore, the use of the mean SID Ca coefficients or previously published coefficients were used to estimate the digestible Ca requirements for broilers from hatch to d 10 (David et al., 2021; Walk et al., 2021b). Formulating

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Table 1. Calculated standardized ileal digestibility (SID) of Ca in various feed ingredients.

Ingredient	Total Ca, %	SID of Ca, %[1]
Wheat	0.04	71.0
Corn dried distillers grains with solubles	0.08	70.0
Corn	0.05	70.0
Soybean meal	0.37	54.0
Canola meal	0.71	31.0
Limestone[2]	37.86	56.0
Dicalcium phosphate	19.93	42.0

[1]Mean values presented in Walk et al. (2021a).
[2]Limestone particle size and in vitro solubility at 15 and 30 min were measured and the SID Ca coefficient for limestone was predicted using the methods of Kim et al. (2019).

diets to meet a digestible Ca requirement may help to mitigate excess total dietary Ca in the final diet in 2 ways: 1) improve our knowledge and understanding of the Ca contribution from the different feed ingredients and feed additives and 2) mitigate the over- or under-supply Ca from ingredients because we have assigned a Ca digestibility coefficient to that specific ingredient in our feed formulation system. For example, the Ca digestibility coefficient for limestone might differ depending on the particle size, in vitro solubility, and if phytase is used in the diets (Kim et al., 2018; Kim et al., 2019). Whereas, the Ca digestibility coefficient for animal by-product meals may differ depending on the nutrient composition, particle size, and proportion of bone and soft tissue (Anwar et al., 2015). Furthermore, the digestible Ca coefficients used for specific ingredients may also differ depending on the broiler age. For example, David et al. (2020) estimated the true ileal Ca digestibility of soybean meal or canola meal was 0.51 or 0.53, respectively, in 21-d-old broilers and this was decreased to 0.33 or 0.22, respectively in 42-d-old broilers.

Understanding the factors that influence the digestibility coefficients, assigning these values in feed formulations, and formulating diets to meet digestible Ca requirements should improve growth rate, skeletal development and nutrient utilization. Therefore, the objective of this work was to use previously published SID Ca coefficients (Table 1) and 3 different nonlinear models to estimate the SID Ca requirement of broilers from d 11 to 24 post-hatch. Due to the impact of particle size, in vitro solubility, and phytase, the SID Ca coefficient for the limestone used in the current study was estimated using methods of Kim et al. (2019). Finally, because dietary phytate can influence Ca digestibility, phytase was supplemented at levels expected to create nearly 'phytate-free' diets.

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 Animal Nutrition Research Center Co., Ltd (Bazhou, China).

Five-hundred and seventy-six Arbor Acres Plus male broilers were obtained on the d of hatch and randomly allocated to 96 cages with 6 birds per cage. From hatch to d 24, sixteen randomly selected cages of birds were fed a nutrient adequate diet formulated using total Ca and the exact same ingredients as all other experimental diets. This diet served as the reference diet to compare with the experimental diets formulated using SID Ca. The reference diet contained 0.96% total Ca and 0.48% avP in the starter phase (hatch to d 10; Table 2) and

Table 2. Ingredient and analyzed nutrient content of the starter experimental diets, as-fed basis.

Ingredient, %	0.50% SID[1] Ca	Reference diet
Wheat	10.00	10.00
Corn dried distillers grains with solubles	5.00	5.00
Corn	46.50	42.33
Soybean meal	29.15	31.27
Canola meal	3.50	3.50
Soybean oil	1.60	3.21
Salt	0.32	0.32
Limestone	1.50	0.55
Dicalcium phosphate	1.15	2.55
Sodium bicarbonate	0.10	0.10
Lysine HCl	0.27	0.26
DL-Methionine	0.27	0.30
Threonine	0.09	0.12
Premix[2]	0.50	0.50
Protease[3]	0.02	0.00
Phytase[4]	0.03	0.00
Carbohydrase[5]	0.01	0.00
Formulated nutrient composition, %		
CP	23.00	23.00
ME, kcal/kg	3,000.00	3,000.00
DM	88.91	89.14
Total Ca	1.03	0.96
SID Ca	0.50	0.42
P	0.60	0.82
Available P	0.48	0.48
Non-phytate P	0.55	0.55
Phytate P	0.27	0.27
Digestible methionine + cystine	0.95	0.95
Digestible lysine	1.28	1.28
Digestible threonine	0.86	0.86
Na	0.18	0.18
Cl	0.30	0.30
Analyzed nutrient composition, %		
Total Ca	0.93	0.95
Total P	0.59	0.81
Phytate P	0.23	0.23
Enzyme activity recovered		
Protease, PROT/kg	15,606	291
B-glucanase, FBG/kg	22	12
Xylanase, FXU/kg	504	57
Phytase, FYT/kg	3,558	33

[1]Standardized ileal digestible.
[2]Vitamin and mineral premix provided (per kilogram of starter diet): vitamin A 12,000 IU; vitamin D_3 2,240 IU; 25-OH-D_3 69 μg; vitamin E 150 IU; vitamin K_3 3 mg; vitamin B_1 3 mg; vitamin B_2 8 mg; vitamin B_6 4 mg; vitamin B_{12} 0.02 mg; biotin 0.25 mg; folic acid 2 mg; niacinamide 60 mg; D-pantothenic acid 15 mg; Fe (as $FeSO_4$) 40 mg; Cu (as $CuCl_2$) 15 mg; Mn (as $MnSO_4$) 110 mg; Zn (as $ZnSO_4$) 90 mg; I (as KIO_3) 0.5 mg; Se (as Na_2SeO_3) 0.25 mg; choline (as choline chloride) 400 mg.
[3]Ronozyme ProAct, CT (DSM Nutritional Products, Kaiseraugst, Switzerland) with an expected activity of 75,000 PROT/g, contributed 0.67% crude protein, 0.04% digestible methionine + cysteine, 0.05% digestible lysine, and 0.05% digestible threonine.
[4]Ronozyme HiPhos GT (DSM Nutritional Products, Kaiseraugst, Switzerland) with an expected activity of 10,000 FYT/g, contributed 0.19% available P.
[5]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.

Table 3. Ingredient and nutrient content of the grower experimental diets, as-fed basis.

Ingredient, %	Standardized ileal digestible calcium, %		Reference diet
	0.56	0.16	
Wheat	10.00	10.00	10.00
Corn dried distillers grains with solubles	5.00	5.00	5.00
Corn	47.88	51.63	44.67
Soybean meal	25.96	25.26	27.90
Canola meal	3.50	3.50	3.50
Soybean oil	3.11	1.95	4.43
Salt	0.32	0.32	0.32
Limestone	1.93	0.04	0.50
Dicalcium phosphate	0.85	0.84	2.25
Sodium bicarbonate	0.10	0.10	0.10
Lysine HCl	0.21	0.22	0.21
DL-Methionine	0.23	0.22	0.25
Threonine	0.05	0.05	0.08
Premix[1]	0.50	0.50	0.50
Protease[2]	0.02	0.02	0.00
Phytase[3]	0.03	0.03	0.00
Carbohydrase[4]	0.01	0.01	0.00
Titanium dioxide	0.30	0.30	0.30
Formulated nutrient composition, %			
CP	21.50	21.50	21.50
ME, kcal/kg	3,100.00	3,100.00	3,100.00
DM	88.83	88.53	88.99
Total Ca	1.13	0.41	0.87
SID Ca	0.56	0.16	0.38
P	0.54	0.54	0.76
Available P	0.44	0.44	0.44
Non-phytate P	0.50	0.50	0.50
Phytate P	0.26	0.27	0.26
Digestible methionine + cystine	0.87	0.87	0.87
Digestible lysine	1.15	1.15	1.15
Digestible threonine	0.77	0.77	0.77
Na	0.18	0.18	0.18
Cl	0.29	0.30	0.29

[1]Vitamin and mineral premix provided (per kilogram of grower diet): vitamin A, 10,000 IU; vitamin D_3, 2,240 IU; 25-OH-D_3, 69 μg; vitamin E, 150 IU; vitamin K_3, 3 mg; vitamin B_1, 2 mg; vitamin B_2, 7 mg; vitamin B_6, 4 mg; vitamin B_{12}, 0.02 mg; biotin, 0.25 mg; folic acid, 2 mg; niacinamide, 60 mg; D-pantothenic acid, 12 mg; Fe (as $FeSO_4$), 40 mg; Cu (as $CuCl_2$), 15 mg; Mn (as $MnSO_4$), 110 mg; Zn (as $ZnSO_4$), 90 mg; I (as KIO_3), 0.5 mg; Se (as Na_2SeO_3), 0.25 mg; choline (as choline chloride), 400 mg.

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

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

[4]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.

0.87% total Ca and 0.44% avP in the grower phytase (d 11 to 24; Table 3). There were no exogenous enzymes added to the reference diet.

The remaining 80 cages were fed a nutrient adequate diet, formulated to contain 0.50% SID Ca (1.03% total Ca) and 0.48% avP from hatch to d 10. On d 11, five diets containing 0.56, 0.46, 0.36, 0.26, or 0.16% SID Ca were assigned to 16 cages per diet in a complete randomized block design. Available P, defined as the part of dietary total P that, at marginal levels of dietary P, can be utilized to cover the P requirement (Shastak and Rodehutscord, 2015) was maintained at 0.48% from hatch to d 10 and 0.44% from d 11 to 24, including 0.19% avP from 3,000 FYT/kg of phytase per the manufacture's recommendations (Ronozyme HiPhos GT, DSM Nutritional Products, Kaiseraugst, Switzerland) in the SID Ca diets. To ensure graded concentrations of SID Ca were achieved, a large batch of the 0.56 and 0.16% SID Ca diets were mixed and then blended at 75:25, 50:50, or 25:75 ratios to create the 0.46, 0.36, or 0.26% SID Ca diets, respectively. The SID Ca diets also contained xylanase (Ronozyme Multigrain, DSM Nutritional Products, Kaiseraugst, Switzerland) and protease (Ronozyme ProAct CT, DSM Nutritional Products) at the expense of energy, protein and amino acids.

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 (Arbor Acres Plus Broiler Pocket Guide, 2018, Huntsville, AL). Birds were allowed ad libitum access to feed and water. At placement (d 0), on d 11 and d 24, all birds were weighed by cage to determine mean BW and calculate mean BW gain (**BWG**). Feed addition and feed left were weighed at d 0, 10, and 24 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 for mortality according to the number of bird days per cage.

From d 21 to 24, excreta was collected daily from 12 replicate cages per treatment, pooled within cage, and frozen until further analyses. On d 24 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) were collected by flushing with distilled water, pooled within cage, and immediately frozen. Left tibias were obtained from 2 birds, close to the 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). Calcium, P, and Ti were then used to determine apparent ileal digestibility (**AID**) and nutrient retention. Tibias were stripped of adhering tissues, fat was extracted using ethanol and petroleum ether for 20 h each, the tibias were dried at 105°C for 24 h and then ashed at 550°C for 48 h 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. 16.0 (SAS Institute, Cary, NC). Cages were divided into 16 blocks with 6 cages (treatments) per block in a complete randomized block design. 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 (**RMSE**) plus or minus the mean of the response, were removed from the statistical analysis. Growth performance and livability in the starter phase (hatch to d 10) were analyzed using the fit Y by X platform, due to the unequal variances between the treatments. For the grower phase (d 11 to 24), 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 or 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 at 100% (or the maximum value) of the requirement using various methods of nonlinear regression in JMP Pro v. 16.0 (SAS). The models included polynomial (quadratic) and straight-broken line (**SBL**) or quadratic-broken line (**QBL**) regressions. The statistical model with the greatest R-square and the lowest RMSE was used to estimate the requirement. Significance was accepted at $P < 0.05$ for all statistical evaluations.

RESULTS

Formulated and analyzed total Ca, P, and enzyme activities were as expected in the starter (Table 2) or grower diets (Table 4) when considering analytical variation and overages in the enzyme premix. Initial BW was 46.8 ± 0.2 g and not influenced by diet ($P = 0.76$). Overall, livability was 94.5% and not influenced by diet from hatch to d 10 or d 11 to 24 post-hatch (Tables 5 and 6). One cage of birds fed 0.16% SID Ca and one cage of birds fed the reference diet, both block 12, were considered outliers and removed from the growth performance statistical analyses. One cage of birds fed 0.46% SID Ca and one cage of birds fed 0.26% SID Ca, both block 10, were considered outliers and removed from the AID Ca statistical analyses.

Table 4. Analyzed nutrient content and enzyme activities recovered in the experimental diets, as-fed basis[1].

	Standardized ileal digestible calcium, %					
Item	0.56	0.46	0.36	0.26	0.16	Reference diet
Analyzed nutrients, %						
DM	89.5	89.3	89.1	89.2	89.3	88.7
Total Ca	1.03	0.88	0.71	0.56	0.37	0.80
Total P	0.55	0.55	0.54	0.53	0.53	0.76
Phytate P	0.23	0.23	0.23	0.24	0.24	0.23
CP	20.5	20.3	20.5	20.2	20.5	21.0
Total lysine	1.04	1.02	1.03	1.03	1.05	1.07
Total methionine	0.54	0.57	0.52	0.50	0.54	0.56
Total cysteine	0.33	0.33	0.31	0.33	0.31	0.34
Total threonine	0.81	0.80	0.80	0.80	0.80	0.86
Enzyme activities recovered						
Protease, PROT/kg	16,191	15,553	17,329	17,043	16,789	490
B-glucanase, FBG/kg	20	20	19	18	17	1
Xylanase, FXU/kg	234	237	190	187	239	0
Phytase, FYT/kg	3,582	3,758	3,208	3,347	3,486	72

[1]Diets were analyzed in duplicate.

Table 5. Growth performance of broilers fed nutrient adequate diets formulated using total or standardized ileal digestible (SID) Ca requirements from hatch to 10 d post-hatch[1].

Experimental diet	Feed intake, g	BW gain, g	mFCR[2], g:g	Livability, %
Reference diet[3]	243	236	1.030	96.9
SEM	4.5	4.0	0.01	1.9
0.50% SID Ca	252	250	1.009	96.0
SEM	2.0	1.8	0.00	0.8
Model *P*-value	0.0637	0.0009	0.0744	0.6853

[1]Data are least square means of 6 birds per cage and 16 or 80 replicate cages for the reference diet or the SID Ca diet, respectively.
[2]Mortality corrected feed conversion ratio.
[3]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 and without exogenous enzymes.

From hatch to d 10 post-hatch, birds fed the diet formulated using SID Ca gained more ($P < 0.05$) compared with birds fed diets formulated using total Ca (Table 5). During the grower phase (d 11 to 24) there was no effect of diet or graded levels of SID Ca on FI, BWG or livability (Table 6). Mortality corrected FCR was numerically lower in birds fed 0.36 or 0.26% SID Ca and increased at all other concentrations of SID Ca, resulting in a quadratic ($P < 0.05$) effect of SID Ca on FCR. In addition, birds fed 0.46 or 0.16% SID Ca had greater ($P < 0.05$) FCR compared with birds fed the reference diet.

Tibia ash percent was numerically higher in birds fed 0.56, 0.46, or 0.36% SID Ca and decreased as SID Ca in the diet decreased to 0.26 or 0.16% SID Ca (quadratic, $P < 0.05$) and this was lower ($P < 0.05$) than birds fed the reference diet (Table 6). Apparent ileal digestibility of Ca (quadratic, $P < 0.05$), AID of P (linear, $P < 0.05$) and digested P (linear, $P < 0.05$) were numerically greater in birds fed low concentrations of SID Ca and decreased as the SID Ca content in the diet increased to 0.56% (Table 7). In addition, birds fed 0.36 to 0.16 or 0.46 to 0.16% SID Ca had greater ($P < 0.05$) AID of Ca or P, respectively, compared with birds fed the reference diet. Digested P was lower ($P < 0.05$) or greater ($P < 0.05$) in birds fed 0.56 to 0.36% or 0.16% SID Ca, respectively compared with birds fed the reference diet. Digested Ca was greater ($P < 0.05$) in birds fed 0.56 or 0.46% SID Ca and lower ($P < 0.05$) in birds fed 0.16% SID Ca when compared with birds fed the reference diet. Apparent Ca retention linearly ($P < 0.05$) increased and apparent N retention linearly ($P < 0.05$) decreased as the SID Ca content in the diet decreased from 0.56 to 0.16%; whereas apparent P retention was numerically higher in birds fed 0.46 or 0.36% SID Ca and then decreased as the SID Ca increased or decreased (quadratic, $P < 0.05$). Apparent P retention was greatest ($P < 0.05$) in birds fed all diets formulated using SID Ca compared with birds fed the reference diet. Apparent Ca retention was lower ($P < 0.05$) or greater ($P < 0.05$) in birds fed 0.56 or 0.36 to 0.16% SID Ca, respectively, compared with birds fed the reference diet. Apparent N retention was lower ($P < 0.05$) in birds fed 0.16% SID Ca compared with birds fed the reference diet.

The SID Ca requirement was estimated at 100% or using the maximum value (quadratic). For tibia ash percent, apparent P retention, or AID of Ca the prediction model with the greatest R^2 and lowest RMSE was the quadratic, QBL, or QBL, respectively (Table 8). The SID Ca requirement for Arbor Acres Plus male broilers from d 11 to 24 was estimated at 0.515 (Figure 1), 0.488 (Figure 2), or 0.380% (Figure 3) when using tibia ash percent, AID of Ca, or apparent P retention,

Table 6. Growth performance and tibia ash of broilers fed graded levels of standardized ileal digestible (SID) Ca from d 11 to 24 post-hatch[1].

SID Ca, %	Feed intake, g	BW gain, g	mFCR[2], g:g	Livability, %	Tibia ash[3], %
0.56	1,175	855	1.376	100.0	45.90
0.46	1,187	861	1.380*	100.0	45.82
0.36	1,197	883	1.357	99.0	45.11
0.26	1,160	862	1.347	95.4	44.43**
0.16	1,192	873	1.377*	97.9	42.50***
Reference diet[4]	1,174	879	1.346	99.0	46.76
SEM	16	14	0.008	1.2	0.46
P-value					
Diet[5]	0.6177	0.6873	0.0050	0.0813	< 0.0001
Linear SID Ca	-	-	0.0086	-	< 0.0001
Quadratic SID Ca	-	-	0.0076	-	0.0360
Block	0.0415	0.1197	0.0100	0.6017	0.1453

[1]Data are least square means of 6 birds per cage and 15 or 16 replicate cages per treatment.
[2]Mortality corrected feed conversion ratio.
[3]Data are least square means of left tibias obtained from 2 birds close to the average body weight per cage, pooled, and 12 replicate cages per treatment.
[4]The reference diet was formulated to meet or exceed nutrient requirements for fast growing broilers using 0.87% total calcium and 0.44% available P and without exogenous enzymes.
[5]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). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$.

Table 7. Calcium and P utilization of 24-day-old broilers fed graded levels of standardized ileal digestible (SID) Ca from d 11 to 24 post-hatch[1].

	Apparent ileal digestibility		Apparent ileal digestible[2]		Apparent retention (excreta)		
SID Ca, %	Ca, %	P, %	Ca, %	P, %	Ca, %	P, %	N, %
0.56	46.57	58.73	0.48***	0.32***	40.02***	60.98***	65.39
0.46	49.99	66.71***	0.44**	0.36***	48.78	62.26***	64.90
0.36	58.54***	70.18***	0.42	0.38**	60.69***	63.78***	65.27
0.26	70.67***	78.59***	0.39	0.41	70.99***	59.62***	63.76
0.16	79.44***	85.63***	0.30***	0.45**	79.54***	52.44***	62.48*
Reference diet[3]	47.12	55.06	0.38	0.42	52.52	46.36	64.69
SEM	1.4	1.2	0.01	0.01	1.0	0.8	0.5
P-value							
Diet[4]	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0019
Linear SID Ca	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0001
Quadratic SID Ca	0.0086	0.5034	0.0149	0.4011	0.5966	< 0.0001	0.0852
Block	0.1746	0.0003	0.1727	0.0006	0.7460	0.4462	0.1312

[1]Data are least square means of pooled digesta contents obtained from all remaining birds per cage and 11 or 12 replicate cages per treatment, presented on an as is basis.

[2]Analyzed total dietary nutrient × apparent ileal nutrient digestibility.

[3]The reference diet was formulated to meet or exceed nutrient requirements for fast growing broilers using 0.87% total calcium and 0.44% available P and without exogenous enzymes.

[4]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). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$.

Table 8. Comparison of the prediction models and the estimated standardized ileal digestible (SID) Ca requirement of broilers from d 11 to 24 post-hatch.

Nonlinear regression model	R^2	Root mean square error	*P*-value	Estimated SID Ca requirement, %
Tibia ash, %				
Quadratic[1]	0.360	1.693	0.0453	0.515
Straight-broken line	0.342	1.717	< 0.05	0.461
Quadratic-broken line	0.352	1.719	< 0.05	0.394
Apparent P retention, %				
Quadratic[1]	0.647	2.939	< 0.001	0.420
Straight-broken line	0.602	3.123	< 0.05	0.357
Quadratic-broken line	0.659	2.918	< 0.05	0.380
Apparent ileal digestibility of Ca, %				
Quadratic[1]	0.859	5.163	0.0139	> 0.56
Straight-broken line	0.864	5.070	< 0.05	0.488
Quadratic-broken line	0.864	5.069	< 0.05	0.488

[1]Estimated using the maximum response.

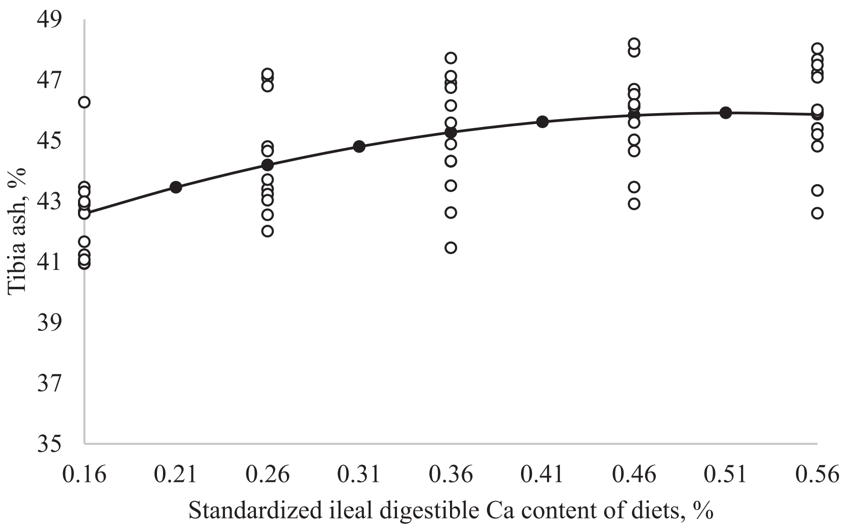

Figure 1. Estimated (•) and measured (○) tibia ash percent of 24-d-old broilers fed graded concentrations of standardized ileal digestible (SID) Ca from d 11 to 24 post-hatch. The SID Ca requirement was estimated at 0.515 (maximum value) using a quadratic regression ($38.936 + 27.020 * SID\ Ca, \% - 26.155 * SID\ Ca, \%^2$; $R^2 = 0.36$; root mean square error = 1.69; $P = 0.0453$).

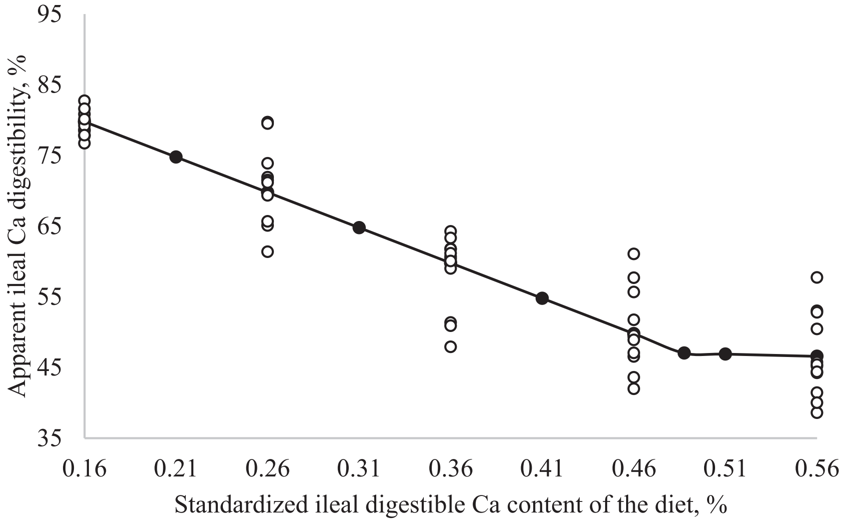

Figure 2. Estimated (•) and measured (○) apparent ileal Ca digestibility of 24-d-old broilers fed graded concentrations of standardized ileal digestible (SID) Ca from d 11 to 24 post-hatch. The SID Ca requirement was estimated at 0.488% using a profiler graph and a straight-broken line regression [$44.24 + 88.16 * (R - x)$, where $(R - x)$ is defined as zero when $x > R$, *else* $(x < R) * (R - x)$; R = 0.55; $R^2 = 0.86$; root mean square error = 5.070; $P < 0.05$].

respectively. This corresponds to a SID Ca to available P ratio of 1.17 to 0.86.

DISCUSSION

The SID Ca coefficients for the main ingredients used in the experimental diets, with the exception of limestone, were obtained from a review article (Walk et al., 2021a). 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 considers the physiological properties of the limestone source, including particle size and in vitro solubility at 15 and 30 min and was a good predictor of in vivo Ca digestibility (adjusted $R^2 = 0.98$; Kim et al., 2019).

From hatch to d 10 post-hatch, birds fed the diet formulated using SID Ca gained more compared with birds fed diets formulated using total Ca and this agrees with a previous study evaluating the SID Ca requirement of broilers from hatch to d 10 (Walk et al., 2021b). During the grower phase (d 11 to 24) there was no effect of diet or graded concentrations of SID Ca on FI or BWG. Similar results were also reported in broilers fed graded concentrations of SID Ca from hatch to d 10 (Walk et al., 2021b). However, David et al. (2021) reported significant decreases in broiler BWG as SID Ca content in the diet increased from 0.33 to 0.55% when broilers were fed diets formulated to contain 0.34% non-phytate P. The negative impact of increasing SID Ca was mitigated as the non-phytate P content of the diet increased from 0.34 to 0.64% (David et al., 2021). In the current study, the lack of any effect of graded levels of dietary SID Ca on BWG or FI was most likely associated with the P levels in the diets, which were formulated to meet or exceed nutrient requirements.

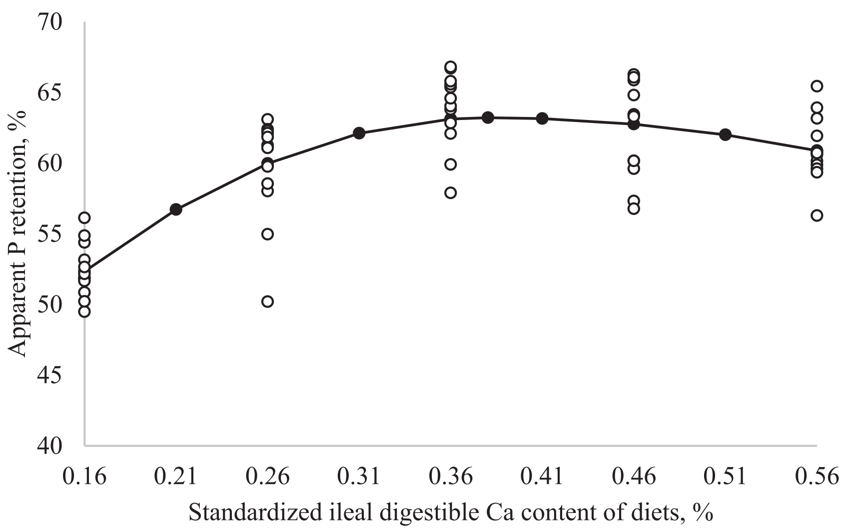

Figure 3. Estimated (•) and measured (○) apparent P retention of 24 d-old broilers fed graded concentrations of standardized ileal digestible (SID) Ca from d 11 to 24 post-hatch. The SID Ca requirement was estimated at 0.38% using a profiler graph and a quadratic-broken line regression [$63.216 - 224.09 *(R - x) - 72.236 * (R - x) * (R - x)$, where $(R - x)$ is zero at values of $x > R$; $R = 0.38$ $R^2 = 0.66$; root mean square error = 2.92; $P < 0.05$].

Tibia ash percent responded quadratically to graded levels of SID Ca and was greatest in birds fed 0.56 to 0.36% SID Ca and significantly lower in birds fed 0.26 or 0.16% SID Ca compared with birds fed the reference diet. Walk et al. (2021b) reported significant effects of graded levels of dietary SID Ca on tibia ash percent in the absence of any impact on growth performance in broilers from hatch to d 10. These results further confirm the Ca and P requirement for skeletal development is much greater than those required for growth and this agrees with previous studies in broilers (David et al., 2021; Walk et al., 2021b) or pigs (Gonzalez-Vega et al., 2016). Due to the quadratic relationship between dietary SID Ca and tibia ash percent, the SID Ca requirement to maximize bone ash was estimated between 0.394 and 0.515% depending on the prediction model employed. To our knowledge, this is the first set of data estimating the SID Ca requirement of fast growing broilers from d 11 to 24 and comparison data is not yet available.

In general, the AID of Ca and P and retention of Ca increased as the dietary SID Ca concentration decreased and this was greater than birds fed the reference diet (at the lower concentrations of SID Ca). Previous authors have also reported significant improvements in P digestibility (Amerah et al., 2014; Kim et al., 2018) or P and Ca retention (David et al., 2021; Walk et al., 2021b) as dietary Ca decreased. However, the influence of graded concentrations of dietary SID Ca on the AID of Ca is less consistent compared with P utilization or total tract retention of Ca. For example, David et al. (2021) reported no effect of graded levels of dietary SID Ca on the AID of Ca in broilers from hatch to d 10, but observed significant increases in the apparent total tract retention of Ca as dietary SID Ca decreased. In the current study and a similar study in broilers from hatch to d 10 (Walk et al., 2021b), decreasing SID Ca significantly increased the AID of Ca and also increased the apparent total tract retention of Ca. The differences between the studies may be attributed to the high doses of phytase used in the experimental diets and the SID Ca concentrations employed were decreased to a greater extent in the current study and the study by Walk et al. (2021b) compared to those tested by David et al. (2021). Regardless of the differences in the experimental methods, the use of apparent total tract retention of Ca might be a better indicator of Ca utilization in broilers and better response criteria for 'long-term' broiler studies (i.e., > 48 h).

Three different nonlinear prediction models were employed and compared when estimating the SID Ca requirement of broilers from d 11 to 24. These included the quadratic polynomial model, SBL and QBL regressions. The requirement was estimated at 100% or using the maximum value (quadratic) and the model with the highest R^2 and the lowest RMSE. In all instances, the estimated SID Ca requirement was greater if the quadratic model was employed when compared with estimates from the straight- or quadratic-broken line models. Pesti et al. (2009) reported the quadratic model may overestimate the requirement but is useful to capture toxic effects of over-supplementation of a nutrient compared with a break-point analysis. The detrimental effects of excess dietary Ca on growth performance, especially in P-deficient diets is documented in broilers (Amerah et al., 2014) and pigs (Lagos et al., 2019). Therefore, a quadratic model or a straight- or quadratic-broken line model is useful option to capture both the estimated requirement and any negative effects of over- or under-supply of SID Ca. The disadvantage of the break-point analysis is the need for more nutrient levels (>3) to obtain good estimates (Pesti et al., 2009). In the current study, the R^2 and RMSE was very similar among all three prediction models; and with the exception of tibia ash, the estimated SID Ca requirements to maximize AID of Ca or apparent P retention were similar between the regression models. However, the estimated SID Ca requirement differed depending on the response criteria, with a greater estimated requirement to optimize bone ash percent (0.515; Figure 1) compared with P retention (0.380; Figure 3). Similar results have been previously reported in broilers from hatch to d 10 (David et al., 2021; Walk et al., 2021b) and in growing pigs (Gonzalez-Vega et al., 2016; Lagos et al., 2019). By combining all 3 parameters, the estimated SID Ca requirement for Arbor Acres Plus broilers from d 11 to 24, when fed diets nearly devoid of dietary phytate, was between 0.380 and 0.515%. This corresponds to a SID Ca to available P ratio of 0.86 to 1.17.

Finally, birds fed the reference diet (0.87% total Ca or 0.38% SID Ca) or the diet formulated to contain 0.46% SID Ca had a measured apparent ileal digestible Ca of 0.38 or 0.44%, respectively (Figure 4). Whereas birds fed 0.56% SID Ca decreased AID of Ca and this resulted in a lower measured apparent ileal digestible Ca compared with formulated values. Conversely, birds fed 0.36, 0.26, or 0.16% SID Ca increased AID of Ca and this resulted in a 14, 33, or 47% greater measured apparent ileal digestible Ca compared to what was formulated in the diets. Birds will adapt to Ca and P imbalances by altering digestibility (as measured in the ileum) within approximately 48 h after feeding the imbalanced diets (Angel, 2017). The extent that Ca and P are regulated by the animal will be influenced by 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 which resulted in a deviation from the formulated values. Similar responses were also observed in broilers from hatch to d 10 (Walk et al., 2021b). The lack of a need to upregulate Ca utilization (i.e., no increase digestibility) in birds fed diets containing the reference diet (0.38% SID Ca) or the diet containing 0.46% SID Ca support the estimated SID Ca requirement is between 0.380 and 0.515% for fast-growing broilers from d 11 to 24 post-hatch. The 0.06% absolute increase (0.44 vs 0.38% AID Ca) in the measured ileal digested Ca in birds fed 0.46% SID Ca compared with those fed the reference diet could be attributed to the phytase supplementation in the SID Ca diets. Phytase increased the Ca digestibility of a complete diet by 0.0 to 0.15%

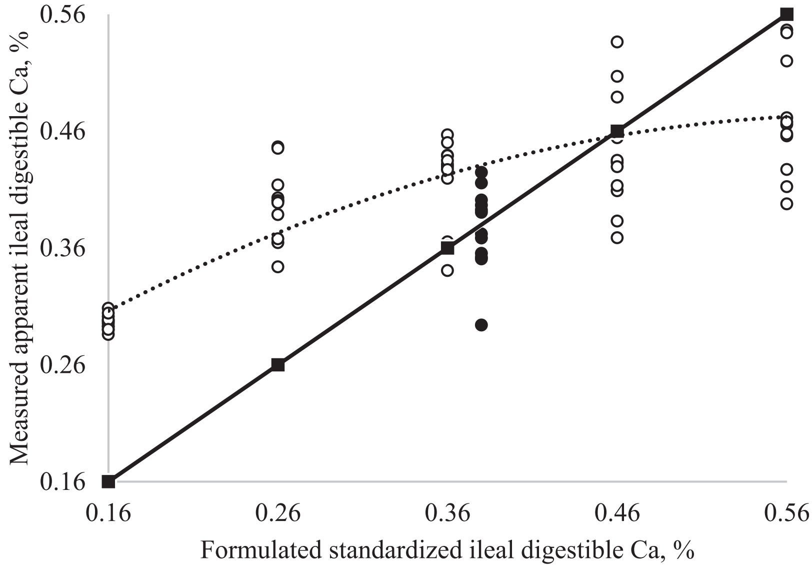

Figure 4. Relationship between the formulated (■) and measured (○) apparent ileal digestible Ca of broilers fed graded concentrations of standardized ileal digestible (SID) Ca from d 11 to 24 post-hatch. The measured SID Ca was 0.48, 0.44, 0.42, 0.39, or 0.30% for broilers fed diets formulated to contain 0.56, 0.46, 0.36, 0.26, or 0.16% SID Ca, respectively. The measured SID Ca of birds fed the PC (•) was 0.38% and this was equivalent to the formulated SID Ca of 0.38% (formulated total Ca of 0.87%).

absolute units when supplemented into diets formulated to contain different levels of dietary total Ca and pulverized (75 um) or particulate (402 um) limestone (Kim et al., 2018). Similarly, Kim et al. (2019) estimated phytase supplementation increased the AID of Ca in a complete diet by an average of 15% relative units and this is similar to the 14% difference reported between the SID Ca (0.44% AID Ca) and reference diet (0.38% AID Ca) in the current study.

To our knowledge, these are the first results evaluating the SID Ca requirement of broilers from d 11 to 24 post-hatch. Formulating broiler diets to meet the SID Ca requirement and using previously published SID Ca coefficients resulted in significant improvements in BWG from hatch to d 10 and significant improvements in P digestibility and retention from d 11 to 24 when compared with broilers fed diets formulated using total Ca and without enzyme supplementation. Bone ash, P retention, and AID of Ca estimate the SID Ca requirement of Arbor Acres Plus male broilers from d 11 to 24 post-hatch is between 0.380 and 0.515%. This corresponds to a SID Ca to available P ratio between 0.86 and 1.17, depending on the response parameter. Three different prediction models were used to estimate the requirements. The quadratic model resulted in the highest estimated requirement compared with the SBL or QBL regressions. The use of quadratic models (polynomials or broken-line) are useful for estimating the SID Ca requirements due to the negative effects noted with under- or over-supplementation of dietary Ca. Repeatability of the current results in diets without or with phytase, the impact of dietary P, and determination of the SID Ca requirements of older birds and of different genetic lines is required to implement SID Ca requirements as standard practice in broiler feed formulations in the future.

DISCLOSURES

The authors declare no conflict of interest.

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