Determination of the standardized ileal digestible calcium requirement of male Arbor Acres Plus broilers from day 25 to 42 post-hatch

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ABSTRACT An experiment was conducted to determine the standardized ileal digestible (**SID**) Ca requirement of Arbor Acres Plus male broilers from d 25 to 42 post-hatch. Broilers were obtained at hatch, placed in floor pens, and fed a nutrient adequate diet until d 24 post-hatch. On d 25, twelve hundred birds were weighed and allocated to one of 4 treatments. There were 25 birds per pen and 12 pens per diet. The diets were formulated to contain 0.46, 0.35, 0.24, or 0.13% SID Ca. Available P (**avP**) was 0.39% in all diets, including 0.16% avP expected from 2,500 FYT/kg of phytase. The SID Ca requirement was estimated using nonlinear models, including quadratic, straight broken-line, and quadratic broken-line. There was no effect of SID Ca on feed intake, body weight gain, feed conversion ratio, or livability. Tibia ash percent was greatest in birds fed 0.35% SID Ca and lowest in birds fed 0.13% SID Ca (quadratic, $P = 0.063$). Apparent ileal digestibility (**AID**) of Ca was highest in birds fed the diets containing 0.13% SID Ca and decreased (quadratic, $P = 0.014$) as dietary SID Ca increased to 0.46%. Apparent digested Ca was highest in birds fed 0.35% SID Ca and lowest in birds fed 0.13% SID Ca (quadratic, $P = 0.005$). Decreasing the concentration of SID Ca in the diet from 0.46 to 0.13% ($P < 0.0001$) increased the AID of P and apparent digested P. Litter N or P were lowest in birds fed 0.35% SID Ca and increased (quadratic, $P \leq 0.05$) as dietary SID Ca decreased to 0.13%. Non-linear equations, developed using tibia ash percent, digested Ca, or litter P, estimate the SID Ca requirement of Arbor Acres Plus broilers from d 25 to 42 was 0.37, 0.35, or 0.35%, respectively. This corresponds to an SID Ca to available P ratio of 0.95 to 0.90.

Key words: broiler, digestible calcium, litter, phosphorus, phytate-free

2022 Poultry Science 101:102146
https://doi.org/10.1016/j.psj.2022.102146

INTRODUCTION

Poultry diets are currently formulated using total Ca and digestible or available P (**avP**). Over supply of dietary total Ca can be detrimental to broiler growth rate (Amerah et al., 2014) and nutrient utilization, including P, amino acids, N, or fat (Amerah et al., 2014; Mutucumarana et al., 2014). This excess total Ca can be the result of a few different factors, such as higher total Ca in ingredients compared to feed formulation values, limestone as a carrier in vitamin and feed additive premixes, and the inclusion of limestone during feed mixing as a macro- rather than a microingredient.

Formulating broiler diets using digestible Ca coefficients and digestible Ca requirements may improve our awareness of ingredients that contain Ca and the quality and subsequent digestibility of the Ca in those ingredients. For example, the digestibility of Ca in limestone can vary depending on particle size and rock-type (Anwar et al., 2016; Kim et al., 2018, 2019). Limestone quality can also vary considerably within and between countries and regions globally (Gilani et al., 2022). Recently, Kim et al. (2019) developed an *in vitro* method to estimate the Ca digestibility of limestone using solubility at 15 and 30 minutes and mean particle size. However, the information for other ingredients that contain 'relatively high' concentrations of Ca, such as inorganic phosphate sources and meat and bone meal is limited and variable.

Considerable efforts have been made to describe the digestible Ca coefficients for ingredients for broilers (Anwar et al. 2017, 2018; David et al. 2019) and understand the impact of limestone quality and phytase on Ca digestibility (Kim et al., 2018, 2019; Li et al., 2021). In a review article evaluating digestible Ca coefficients of

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Received May 20, 2022.
Accepted August 12, 2022.

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ingredients for broilers, Walk et al. (2021b) reported the average ileal Ca digestibility coefficient for limestone, monocalcium phosphate, dicalcium phosphate, meat and bone meal, and a few common cereals. The authors also found there was no significant difference between the apparent ileal digestible (**AID**) or standardized ileal digestible (**SID**) Ca coefficients within an ingredient, but large variation in the ileal Ca digestibility depending on experimental methods, Ca to P ratios in the experimental diets and the presence or absence of phytase.

Considering the limited information available on the SID Ca coefficients for ingredients, a series of studies have been conducted to estimate the SID Ca requirement of fast-growing broilers using mean values from the literature. The requirements have been estimated for broilers from hatch to d 10 (David et al., 2021; Walk et al., 2021b) and d 11 to 24 (Walk et al., 2022a). The previous authors reported the SID Ca requirement to optimize bone ash was greater than that needed to optimize growth, and this was also noted in growing pigs (Gonzalez-Vega et al., 2016). However, the SID Ca requirement for broilers beyond 24 d of age has not been reported. Therefore, the objective of this study was to estimate the SID Ca requirement of broilers from d 25 to 42 post-hatch, when using diets nearly devoid of phytate by including high doses of phytase and an *in vitro* assay to estimate the AID of Ca digestibility of limestone.

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, P. R. China).

Arbor Acres Plus male broilers were obtained on d of hatch and randomly allocated to floor pens on clean litter until d 24 post-hatch. Birds were fed a common, nutrient adequate diet, formulated using previously established SID Ca requirements from hatch to d 10 (Walk et al., 2021b) and d 11 to 24 (Walk et al., 2022a; Table 1). On d 25, birds (n = 1,200) were weighed and randomly allocated to one of four experimental diets. All experimental diets were formulated to meet or exceed Arbor Acres Plus broiler nutrition specifications (Arbor Acres Broiler Nutrition Specifications, Huntsville, AL), except Ca. There were 25 birds per pen and 12 replicate pens per treatment. All birds were reared 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.

The dietary treatments were formulated to contain graded levels of SID Ca at 0.46, 0.35, 0.24, or 0.13%. The ingredient SID Ca coefficients used to create the graded concentrations of SID Ca are listed in Table 2. The limestone used in the experimental diets was analyzed to estimate the Ca digestibility coefficient, including phytase according to methods of Kim et al. (2019). It had a geometric mean diameter of 78 μm and an *in vitro* solubility at 15 and 30 min of 89.68 and 88.73%, respectively. All 4 experimental diets were formulated to contain 0.39% avP, including 0.16% avP from 2,500 FYT/kg phytase per the manufacturer's recommendations (Ronozyme HiPhos GT, DSM Nutritional Products, Kaiseraugst, Switzerland) to mitigate any negative effects of phytate on Ca utilization. The experimental diets also contained an exogenous multi-carbohydrase (Ronozyme Multi-Grain, DSM Nutritional Products) and an exogenous protease (Ronozyme ProAct 360, DSM Nutritional Products) at the expense of 65 kcal/kg of energy and approximately 0.67% crude protein and 0.04 to 0.05% amino acids, respectively, to represent commercial conditions. To ensure graded concentrations of SID Ca were achieved a large batch of the 0.46 and 0.13% SID Ca diets were mixed and then blended at 67:33 or 33:67 ratios to create the 0.35 or 0.24% SID Ca diets, respectively. Titanium dioxide was included in all diets at 0.30%.

Birds were weighed prior to placement (d 25) and d 42 to determine mean BW and calculate mean BW gain (**BWG**). Feed addition and feed left over were weighed at d 25 and 42 to calculate feed intake (**FI**). Body weight gain and FI were used to calculate feed conversion ratio (**FCR**). Mortality was recorded daily. Any culled or dead birds were weighed. Feed intake and subsequently FCR were adjusted according to the number of bird days per pen.

On d 42 after weighing, 4 birds of average BW were euthanized by carbon dioxide asphyxiation. Ileal digesta (defined as the Meckel's diverticulum to 40-mm proximal to the ileocecal junction according to methods of Ravindran et al., 1999) was collected by flushing with distilled water, pooled within pen, and immediately frozen. Left tibias were obtained from 2 birds of average BW per pen and pooled to determine tibia ash. Finally, litter samples were obtained from the same 3 locations within each pen, pooled and frozen until further analyses.

Diets, digesta, and litter were dried to a constant weight, 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). Nitrogen was analyzed using the Dumas Method with a macro-nitrogen analyzer (LECO FP528, LECO Corporation, Miami, FL). Phytic P was determined using a modified method of McKie and McCleary (2016). Briefly, phytate P was extracted by 0.66 N HCl overnight and then followed by the enzymatic hydrolysis of all phosphate residues by a megadose of phytase. Then the free phosphate was measured by a modified colorimetric molybdenum blue assay using an external phosphate standard curve. Phytic P in the diets was calculated as the difference between total P and free P.

Tibias were stripped of adhering tissues, fat was extracted using ethanol and petroleum ether for 20 h each,

Table 1. Calculated ingredient and nutrient content of the experimental diets from hatch to d 42 post-hatch, as-fed.

Ingredient, %	Starter diet D 0–10	Grower diet D 11–24	Standardized ileal digestible (SID) calcium diet	
			0.46%	0.13%
Wheat	10.00	10.00	10.00	10.00
Corn dried distillers grains with solubles	5.00	5.00	5.00	5.00
Corn	43.60	46.69	52.77	56.06
Soybean meal	30.97	27.52	20.35	19.79
Canola meal	3.50	3.50	3.50	3.50
Soya oil	2.35	3.32	4.10	3.07
Salt	0.28	0.29	0.24	0.24
Limestone	1.76	1.56	1.75	0.05
Dicalcium phosphate	1.28	1.00	0.75	0.75
Sodium bicarbonate	0.15	0.15	0.15	0.15
Lysine HCl	0.21	0.16	0.24	0.25
DL-Methionine	0.26	0.21	0.21	0.21
Threonine	0.07	0.03	0.05	0.05
Premix[1]	0.50	0.50	0.50	0.50
Choline chloride	0.06	0.06	0.06	0.06
Protease[2]	0.005	0.005	0.005	0.005
Phytase[3]	0.007	0.007	0.007	0.007
Carbohydrase[4]	0.0075	0.0075	0.0075	0.0075
Titanium dioxide	0.00	0.00	0.30	0.30
Formulated nutrient content, %				
Crude protein	23.00	21.50	18.67	18.67
ME, kcal/kg	3,000	3,100	3,200	3,200
DM	89.04	89.11	88.99	88.72
Total calcium	1.05	0.91	0.90	0.29
SID calcium	0.53	0.46	0.46	0.13
Total phosphorus	0.60	0.53	0.46	0.46
Available phosphorus	0.48	0.44	0.39	0.39
Digestible phosphorus	0.48	0.43	0.38	0.38
Non-phytate phosphorus	0.37	0.31	0.26	0.26
Phytate phosphorus	0.23	0.22	0.20	0.20
Digestible methionine + cystine	0.95	0.87	0.80	0.80
Digestible lysine	1.28	1.15	1.03	1.03
Digestible threonine	0.86	0.77	0.69	0.69
Sodium	0.18	0.18	0.16	0.16
Chloride	0.27	0.26	0.25	0.25

[1] Vitamin and mineral premix provided (per kilogram of diet): vitamin A 9,800 IU; vitamin D_3 3,500 IU; vitamin E 45 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 0.15 mg; folic acid 2 mg; niacinamide 60 mg; D-pantothenic acid 15 mg; Fe (as $FeSO_4$) 50 mg; Cu (as $CuCl_2$) 15 mg; Mn (as $MnSO_4$) 90 mg; Zn (as $ZnSO_4$) 70 mg; I (as KIO_3) 1 mg; Se (as Na_2SeO_3) 0.3 mg.

[2] Ronozyme ProAct 360 (DSM Nutritional Products, Kaiseraugst, Switzerland) with an expected activity of 600,000 PROT/g, contributed 0.67% crude protein, 0.04% methionine + cysteine, 0.05% lysine, and 0.05% threonine.

[3] Ronozyme HiPhos GT (DSM Nutritional Products, Kaiseraugst, Switzerland) with an analyzed activity of 37,974 FYT/g, contributed 0.16% 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.

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.

Table 2. Calculated standardized ileal digestible (SID) calcium coefficients of various feed ingredients.

Ingredient	Total analyzed calcium[1], %	SID of calcium, %[2]
Wheat	0.04	71.0
Corn dried distillers grains with solubles	0.08	70.0
Corn	0.01	70.0
Soybean meal	0.34	54.0
Canola meal	0.72	31.0
Limestone[3]	35.68	54.0
Dicalcium phosphate	21.41	42.0

[1] Total analyzed calcium in each ingredient, analyzed in duplicate.

[2] Mean value presented in Walk et al. (2021a).

[3] Limestone particle size and in vitro solubility at 15 and 30 min were measured and the SID calcium coefficient for limestone was predicted using the methods of Kim et al. (2019).

Statistical Analysis

Data were subjected to an analysis of variance using JMP Pro v. 16.0 (SAS Institute, Cary NC). Pen 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 analyses and the pooled standard error of the mean presented. Growth performance, livability, tibia ash, AID, and litter nutrients were analyzed using the fit model platform. Livability or non-normally distributed data were analyzed using the fit Y by X platform. For all parameters, the statistical model included SID Ca. If SID Ca effects were significant, means were separated using orthogonal linear and quadratic contrast statements. If there was a significant quadratic effect of SID Ca, the SID Ca requirement was estimated using the fit curve (quadratic) or fit nonlinear platform in JMP Pro v. 16 (SAS, Cary NC). The SID Ca requirement was estimated at the maximum value (quadratic) or break point using straight-broken line (**SBL**) or quadratic-broken line (**QBL**) regression models according to methods described by Robbins et al. (2006) and Pesti et al. (2009). Significance was accepted at $P < 0.10$.

RESULTS AND DISCUSSION

Nutrients and enzyme activities recovered in the experimental diets were within formulated ranges when considering analytical variances (Menegat et al., 2019) and product overages (Table 3). The analyzed total Ca concentrations in the diets confirm graded levels of Ca were achieved and each concentration was approximately 0.04 to 0.05 ± 0.015% (absolute value) greater than formulated. These values, including the standard deviation, are well below the 21 to 34% analytical variances associated with total Ca analyses in feed (Menegat et al., 2019), and therefore, the formulated SID Ca levels were used as expected to estimate the SID Ca requirements. Phytase activity recovered in the experimental diets was 99 to 131% of the expected activity

Table 3. Analyzed nutrient content and enzyme activities recovered in the experimental diets[1].

Analyzed nutrients, %	Starter diet	Grower diet	Standardized ileal digestible calcium, %			
			0.46	0.35	0.24	0.13
DM	91.57	91.77	92.53	92.09	92.92	94.11
Total calcium	1.04	0.95	0.94	0.74	0.54	0.34
Total phosphorus	0.60	0.54	0.48	0.46	0.47	0.47
Phytate phosphorus	0.22	0.22	0.20	0.20	0.20	0.20
Crude protein	22.82	21.77	18.96	18.75	19.05	18.85
Enzyme activities recovered						
Protease, PROT/kg	37,741	35,184	33,878	40,515	34,141	39,164
Glucanase, FBG/kg	63	58	57	63	62	76
Xylanase, FXU/kg	278	241	256	297	265	257
Phytase, FYT/kg	2,581	3,017	3,291	2,463	3,072	2,446

[1] Diets were analyzed in duplicate for proximate analysis and triplicate for enzyme activity recoveries.

and this was slightly higher than the acceptable ± 20%. However, this overage would not be expected to influence the current results because all diets were formulated to contain high doses of phytase to degrade nearly all the phytate (Cowieson et al., 2011; Zhang et al., 2022) and there was no effect of diet ($P = 0.351$) on gizzard phytate P concentration (data not shown). Finally, protease recoveries in the experimental diet were greater than expected and this is most likely due to the overages in the product and inclusion level in the diets at commercial recommendations without considering the product overages. There was no effect of the diet ($P = 0.525$) on AID of N (data not shown) and therefore, the differences in recovered protease activity in the experimental diets was not expected to have an influence on the results.

All birds were fed the same nutrient adequate diet from hatch to d 24 and experimental diets were fed from d 25 to 42 post-hatch. Mortality was approximately 2.5 ± 3.2% and not influenced by SID Ca ($P = 0.391$). There was no impact of SID Ca on FI, BWG, or FCR of broilers from d 25 to 42 (Table 4). However, tibia ash percent was greatest in birds fed 0.35% SID Ca and lowest in birds fed 0.13% SID Ca (quadratic, $P = 0.063$; Table 4). Walk et al. (2021b, 2022a) reported significant effects of graded levels of SID Ca on tibia ash in broilers from hatch to d 10 or d 11 to 24 in the absence of any influence of graded levels of SID Ca on growth performance. Furthermore, Rousseau et al. (2012) reported significant effects of graded levels of total Ca (0.37, 0.57, or 0.77%) on tibia ash and bone breaking strength in the absence of any effect on average daily gain or FCR of broilers from d 22 to 38 post-hatch. Birds adapt to dietary excess or deficiencies of Ca through 3 major hormones, parathyroid hormone, calcitonin, and 1,25-dihydroxycholecalciferol and mobilizing Ca through bones and kidneys (Shafey, 1993). In the current study, birds were able to maintain growth performance, especially as the diets were sufficient in avP, through excretion of excess Ca via the kidneys or resorption of Ca from bone in the deficient diets (Shafey, 1993). In addition, the Ca requirement to optimize tibia ash is greater than the requirement to optimize growth performance (Gonzalez-Vega et al., 2016; David et al., 2021; Walk et al., 2021b, 2022a) and Ca is a limiting factor for bone mineralization (Rousseau et al., 2012). It could also be hypothesized that the near complete removal of dietary phytate with high doses of phytase (Cowieson et al., 2011; Ajuwon et al., 2020) improved both Ca and P availability to a greater extent than assumed in the feed formulation, which resulted in limited impacts of the different levels of dietary Ca on growth performance.

The AID of Ca was highest in birds fed 0.13% SID Ca and decreased (quadratic, $P = 0.014$) as the SID Ca content in the diet increased to 0.46% (Table 5). Apparent ileal digested Ca was greatest in birds fed 0.35% SID Ca and decreased (quadratic, $P = 0.005$) as the SID Ca content in the diet decreased to 0.13% (Table 5). The increase in the AID of Ca as the SID Ca content in the

Table 4. Growth performance and tibia ash percent of broilers fed standardized ileal digestible (SID) calcium from hatch to d 24 and graded levels of SID calcium from 25 to 42 d of age[1].

Standardized ileal digestible (SID) calcium, %	Feed intake, g	Body weight gain, g	FCR[2], g: g	Livability, %	Tibia ash, %
0.13	2,853	1,471	1.925	97.6	39.63
0.24	2,878	1,518	1.899	97.9	41.07
0.35	2,854	1,503	1.901	96.2	41.65
0.46	2,837	1,483	1.920	98.3	41.52
Pooled SEM[3]	26	27	0.02	0.91	0.41
P-value					
SID calcium	0.7244	0.6149	0.7277	0.3909	0.0053
Linear	-	-	-	-	0.0018
Quadratic	-	-	-	-	0.0628

[1] Growth performance data are least square means of 25 birds per pen and 11 to 12 replicate pens per treatment. Tibia ash data were obtained on d 42 from 2 birds of average BW pooled per pen.

[2] Mortality corrected feed conversion ratio.

[3] Pooled standard error of the mean.

Table 5. Apparent ileal digestibility of Ca or P of broilers fed standardized ileal digestible (SID) calcium from hatch to d 24 and graded levels of SID calcium from 25 to 42 d of age[1].

Standardized ileal digestible (SID) calcium, %	AID of calcium, %	Apparent ileal digested calcium, %	AID of phosphorus, %	Apparent ileal digested phosphorus, %
0.13	71.91	0.243	82.27	0.387
0.24	52.59	0.282	71.01	0.336
0.35	41.49	0.306	63.30	0.293
0.46	31.13	0.294	53.65	0.258
Pooled SEM[2]	1.21	0.009	0.99	0.005
P-value				
SID calcium	< 0.0001	0.0003	< 0.0001	< 0.0001
Linear	< 0.0001	0.0004	< 0.0001	< 0.0001
Quadratic	0.0136	0.0050	0.4211	0.1622

[1] Data are least square means of pooled digesta contents obtained from 4 birds per pen and 11 to 12 replicate pens per treatment.

[2] Pooled standard error of the mean.

diet decreased resulted in a greater or lower apparent ileal digested Ca compared with what was formulated in the diets (Figure 1). Previous authors have also reported a significant effect of dietary SID Ca level on the AID of Ca (Walk et al., 2021b, 2022a) whereas David et al. (2021) reported no effect of dietary SID Ca level on the AID of Ca, but an increase in SID Ca intake as SID Ca in the diet increased from 0.33 to 0.55%. The current results indicate birds were able to adapt to the dietary Ca deficiencies by increased ileal absorption and possibly reabsorption from the kidneys (Shafey, 1993). This occurred even if the birds were fed a nutrient adequate diet from hatch to d 18 post-hatch and this is in agreement with findings from Yan et al. (2005).

The AID of P or apparent ileal digested P was greatest in birds fed 0.13% SID Ca and decreased (linear, $P < 0.0001$) as the SID Ca content in the diet increased to 0.46%. Previous authors reported significant improvements in the AID of P as dietary total or SID Ca was decreased (Rousseau et al., 2012; Amerah et al., 2014; Walk et al., 2021b, 2022a). Rousseau et al. (2012) suggested the lower Ca diets improve P utilization through a mitigation of insoluble Ca phosphate or Ca-phytate complexes in the small intestine and greater activity of mucosal phytase as reported by Applegate et al. (2003).

Litter dry matter was approximately 59% (Table 6) and lowest in birds fed 0.13% SID Ca and linearly ($P = 0.005$) increased as the SID Ca content in the diet increased to 0.46%. Previous authors reported an increase in litter pH and litter moisture when diets were formulated using SID Ca compared with diets formulated using total Ca, due to a higher total Ca concentration in the SID Ca diets (Walk et al., 2022b). Similar to the current study, the litter dry matter in the study by Walk et al. (2022b) was less than the recommended 70 to 80%, yet there were no incidences of pododermatitis and average litter scores were approximately1.0 in both treatments. These results are difficult to explain and many factors, including environment and husbandry, can influence litter moisture (Ritz et al., 2017). However, the impact of formulating diets using SID Ca on litter moisture should be considered and measured in follow-up studies. Litter N linearly ($P < 0.05$) decreased as the SID Ca concentration in the diet increased to 0.46%. Litter P was lowest in birds fed 0.35% SID Ca and quadratically ($P \leq 0.05$) increased as the SID Ca content in the diet decreased to 0.13% (Table 6). Litter Ca was lowest in birds fed 0.13% SID Ca and increased (quadratic, $P < 0.0001$) as dietary SID Ca increased to 0.46%. Measuring the nutrients excreted in the litter, including P and N is an indication of the utilization of those same nutrients by the bird. In the current study, total litter Ca increased as the SID Ca concentration in the diet increased from 0.13 to 0.46% and this corresponds to the significant decrease in the AID of Ca as the SID Ca

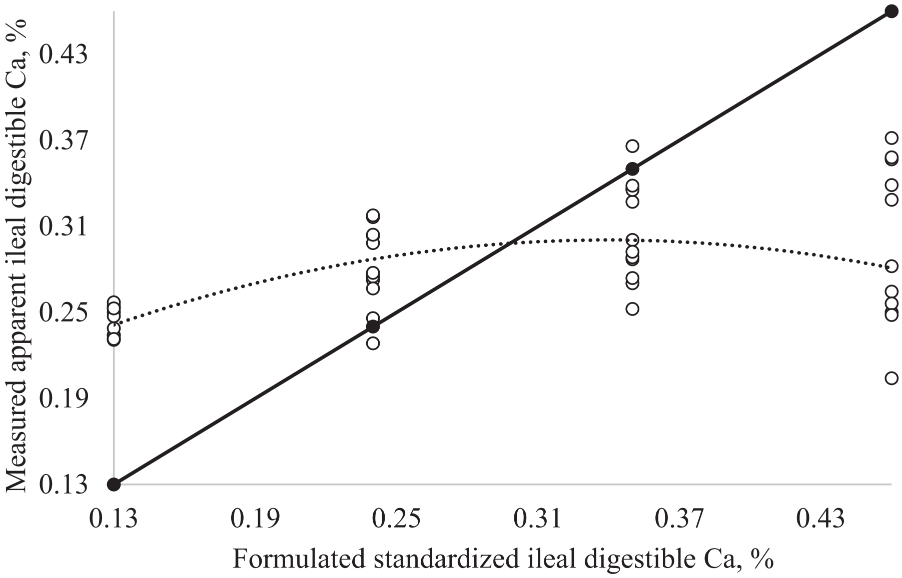

Figure 1. The relationship between the formulated standardized ileal digestible (SID) calcium (x-axis; black closed circles, solid black line) and the measured apparent ileal digestible (AID) calcium (± the pooled standard error of the mean; y-axis; open circles, dashed line) of broilers fed graded concentrations of SID calcium from d 25 to 42 post-hatch. The measured AID of calcium was 0.243, 0.282, 0.306, or 0.294 ± 0.009% for broilers fed diets formulated to contain 0.13, 0.24, 0.35, or 0.46% SID calcium, respectively.

Table 6. Concentration of nitrogen, calcium, or phosphorus in the litter of broilers fed standardized ileal digestible (SID) calcium from hatch to d 24 and graded levels of SID calcium from 25 to 42 d of age[1].

Standardized ileal digestible (SID) calcium, %	Litter DM, %	Litter nitrogen, % DM	Litter calcium, % DM	Litter phosphorus, % DM
0.13	56.69	5.28	1.43	1.52
0.24	57.21	5.14	1.98	1.44
0.35	60.90	4.69	2.44	1.26
0.46	59.52	4.89	3.25	1.32
Pooled SEM[2]	0.98	0.11	0.06	0.04
P-value				
SID calcium	0.0151	0.0038	< 0.0001	< 0.0001
Linear	0.0092	0.0028	< 0.0001	< 0.0001
Quadratic	0.3417	0.1460	0.0330	0.0742

[1] Data are least square means of 11 to 12 replicate pens per treatment.

[2] Pooled standard error of the mean.

concentration in the diet increased. Whereas total P and total N excretion in the litter were lowest in birds fed 0.35% SID Ca which is also very close to the estimated SID Ca requirement (Table 7). These results may indicate formulating diets to optimize Ca utilization may also result in reductions in total P or total N excretion in the litter.

Finally, the SID Ca requirements for broilers from d 25 to 42 post-hatch were estimated using quadratic, SBL, or QBL regressions (Table 7). The response variables included tibia ash percent, AID of Ca, apparent ileal digested Ca, and litter total P. The SID Ca requirement for Arbor Acres Plus male broilers from d 25 to 42 post-hatch was estimated between 0.29 and 0.37% when using tibia ash percent (Figure 2), 0.40 to greater than 0.46% when using the minimum AID of Ca (Figure 3), 0.32 to 0.35% when using apparent ileal digested Ca (Figure 4), or 0.35 to 0.40% when using the lowest concentration of total P in the litter (Figure 5). In general, the quadratic models estimate the requirement at the maximum response, and this resulted in a higher estimated SID Ca requirement compared with and followed by the SBL and then the QBL. However, in most instances the R^2 and RMSE were similar between the models and the estimated requirement ranged from 0.29 to 0.41% SID Ca, depending on the response variable. It is important to note, the data were quite variable for tibia

Table 7. Comparison of the prediction models and the estimated standardized ileal digestible (SID) calcium requirement of broilers from d 25 to 42 post-hatch using different response variables.

Non-linear regression models	Quadratic (maximum value)			Straight-broken line			Quadratic-broken line		
Response variable	R2	RMSE	Est. SID calciumreq. %[1]	R2	RMSE	Est. SID calcium req. %[1]	R2	RMSE	Est. SID calcium req. %[1]
Tibia ash, %	0.27	1.35	0.370	0.27	1.36	0.309	0.27	1.37	0.289
Apparent ileal digestibility of calcium, %[2]	0.91	4.81	> 0.460	0.90	5.05	0.411	0.90	5.05	0.399
Apparent ileal digested calcium, %	0.33	0.04	0.350	0.33	0.04	0.343	0.33	0.04	0.316
Litter total phosphorus, % DM[2]	0.35	0.14	0.420	0.37	0.13	0.351	0.38	013	0.355
Average			0.400			0.354			0.340

[1] The estimated standardized ileal digestible (SID) calcium requirement.

[2] The break point and the estimated requirement was achieved at the minimum response using this parameter.

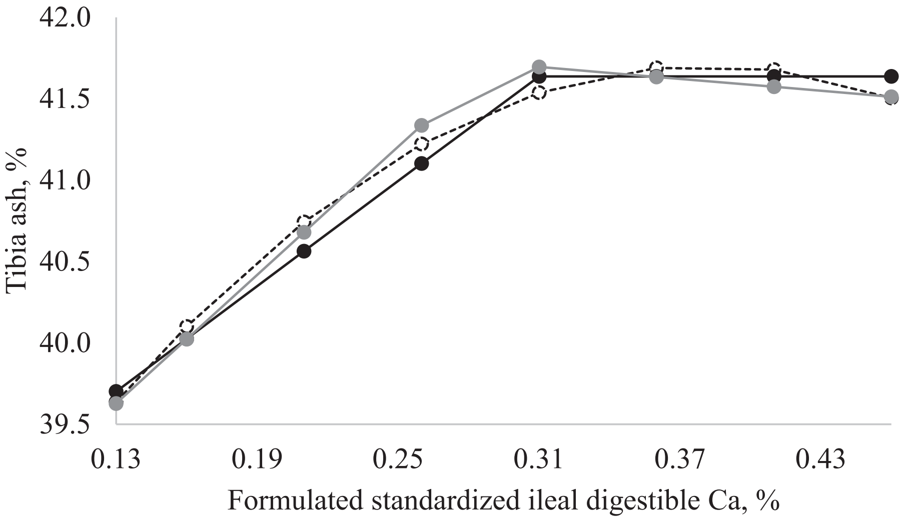

Figure 2. The estimated standardized ileal digestible (SID) calcium requirement (± the standard error) of Arbor Acres Plus male broilers from d 25 to 42 post-hatch using tibia ash percent and three non-linear regression models: quadratic (Q; open circles, dashed line), straight broken-line (SBL; black closed circles, black solid line), or quadratic broken-line (QBL; gray closed circles, gray solid line). The SID calcium requirement was estimated between 0.289 ± 0.06, 0.309 ± 0.06, or 0.370 ± 0.16% using a QBL, SBL, or Q (maximum value) regression, respectively.

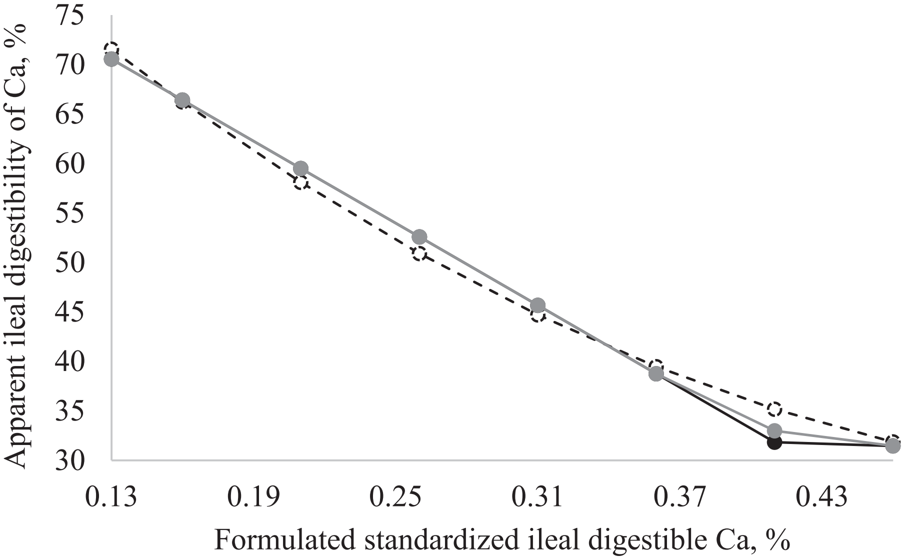

Figure 3. The estimated standardized ileal digestible (SID) calcium requirements (± the standard error) of Arbor Acres Plus male broilers from d 25 to 42 post-hatch using apparent ileal digestibility (AID) of calcium and three non-linear regression models: quadratic (Q; open circles, dashed line), straight broken-line (SBL; black closed circles, black solid line), or quadratic broken-line (QBL; gray closed circles, gray solid line). The SID calcium requirement was estimated between 0.399 ± 0.02, 0.411 ± 0.02, or > 0.460 ± 0.06% using a QBL, SBL, or Q (minimum value) regression, respectively.

ash percent and apparent ileal digested Ca and the SID Ca content in the diet only explained approx. 30% of the response. In addition, the variation in the apparent ileal digested Ca appears to increase as the SID Ca content in the diet increased (Figure 1). For example, birds fed 0.13% SID Ca had a mean apparent ileal digested Ca of 0.24% Ca and a range of 0.23 to 0.26%; whereas birds fed 0.46% SID Ca had a mean apparent ileal digested Ca of 0.29% Ca and this ranged from 0.20 to 0.37%. The increase in variation in the high SID Ca diets and greater apparent ileal digested Ca in the low SID Ca diets both contributed to the poor relationship ($R^2 = 0.24$) between the formulated and measured digested Ca reported in this study. There were no differences in AID of DM ($P = 0.169$) between the experimental diets (data not shown). However, ileal phytate P content linearly ($P < 0.0001$) increased from 0.09, 0.16, 0.17, to 0.22% as the SID Ca content of the diet increased from 0.13, 0.24, 0.35, to 0.46%, respectively. The increase in the phytate P content in the ileal digesta, combined with the greater concentrations of Ca, may have promoted insoluble Ca-phytate complexes in the small intestine (Selle et al., 2009) contributing to the greater variation noted in the apparent ileal digested Ca as the SID Ca content of the diet increased, even in the presence of high doses of phytase.

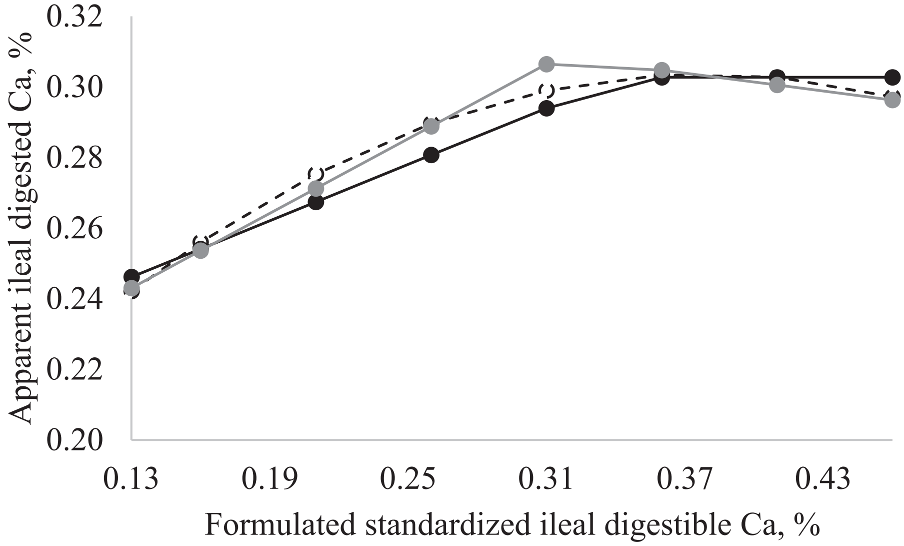

Figure 4. The estimated standardized ileal digestible (SID) calcium requirements (± the standard error) of Arbor Acres Plus male broilers from d 25 to 42 post-hatch using apparent ileal digested calcium and three non-linear regression models: quadratic (Q; open circles, dashed line), straight broken-line (SBL; black closed circles, black solid line), or quadratic broken-line (QBL; gray closed circles, gray solid line). The SID calcium requirement was estimated between 0.316 ± 0.05, 0.343 ± 0.05, or 0.350 ± 0.04% using a QBL, SBL, or Q (maximum value) regression, respectively.

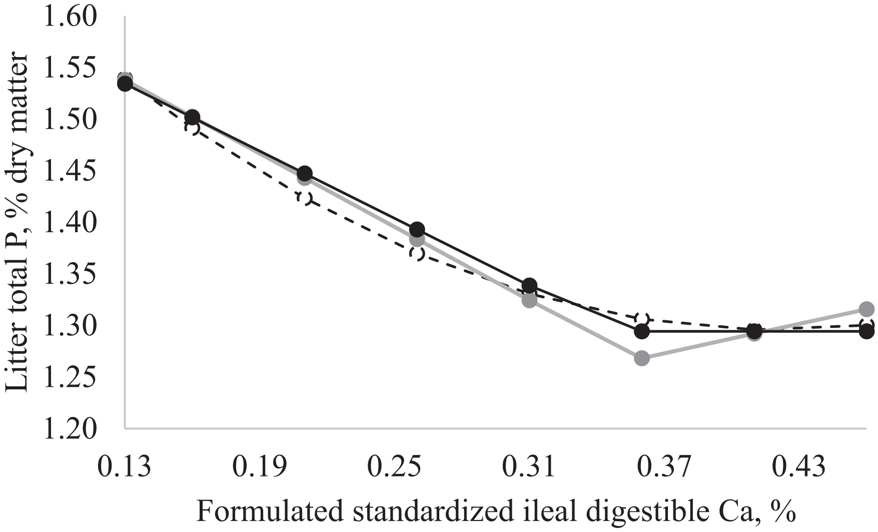

Figure 5. The estimated standardized ileal digestible (SID) calcium requirements (± the standard error) of Arbor Acres Plus male broilers from d 25 to 42 post-hatch using the lowest total P concentration in the litter and three non-linear regression models: quadratic (Q; open circles, dashed line), straight broken-line (SBL; black closed circles, black solid line), or quadratic broken-line (QBL; gray closed circles, gray solid line). The SID calcium requirement was estimated between 0.340 ± 0.03, 0.354 ± 0.03, or 0.400 ± 0.05% using a QBL, SBL, or Q (minimum value) regression, respectively.

In conclusion, three different experimental models were used to estimate the SID Ca requirement of male Arbor Acres Plus broilers from d 25 to 42 post-hatch. The quadratic models estimated the requirement at the maximum response, and this resulted in a higher estimated SID Ca requirement, followed by the SBL and then the QBL. Tibia ash, AID of Ca, apparent ileal digested Ca, or litter total P were used to estimate the SID Ca requirement between 0.29 and 0.41%, depending on the response variable and the statistical model. This corresponds to a SID Ca to avP ratio between 0.74 and 1.05 for broilers from d 25 to 42 post-hatch.

DISCLOSURES

The authors declare no conflict of interest.

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