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Original Research Article

# Determination of the standardized ileal digestible calcium requirement of Ross broilers from hatch to day 14 post-hatch

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A B S T R A C T

An experiment was conducted to determine the standardized ileal digestible (SID) calcium requirement of fast-growing broilers from hatch to d 14 post-hatch. Ross 308 ($n = 360$) male broilers were obtained on day of hatch and allocated to 1 of 5 treatments in battery cages. There were 6 birds per cage and 12 pens per treatment. Four treatments were formulated to contain 0.60%, 0.46%, 0.32% or 0.18% SID Ca. The final treatment was formulated using total Ca to meet or exceed all nutrient requirements, including 0.90% total Ca and 0.49% non-phytate P (nPP), using the same ingredients. This treatment was the reference diet for comparison and validation of the SID Ca diets. Birds and feed were weighed at placement and on d 14. Tibias and ileal contents were obtained on d 14 and excreta was collected per pen and pooled on d 14. Data were analyzed using JMP Pro and requirements were estimated using 3 different non-linear regression models. Increasing the SID Ca content in the diet from 0.18% to 0.60% improved (quadratic, $P < 0.05$) body weight gain and mortality corrected feed conversion ratio (mFCR). The estimated SID Ca requirement to optimize gain or mFCR was between 0.39% and 0.52%. Tibia ash percent and weight increased (quadratic, $P < 0.05$) as SID Ca content in the diet increased and the estimated SID Ca requirement was between 0.32% and 0.58%. Phosphorus utilization was improved in birds fed diets formulated using SID Ca compared with birds fed the reference diet. In conclusion, the SID Ca requirement of fast-growing broilers from hatch to d 14 was estimated between 0.534% and 0.398% when quadratic, straight-broken line, or quadratic-broken line regressions were used. These results agree with previously published data evaluating the SID Ca requirement of fast-growth broilers from hatch to d 10.



## 1. Introduction

Estimating the total Ca requirement and optimizing dietary Ca for meat-type, broiler chickens is not a new concept. In 1961, Simco and Stephenson (1961) estimated the total Ca requirement to optimize gain, feed to gain, and toe ash could be as low as 0.50%. Whereas, in 1963, Edwards Jr. and colleagues estimated the total Ca requirement to optimize gain and feed to gain was between 0.99% and 1.07% and much greater at 1.39% to 1.47% total Ca to optimize bone ash. The differences in the estimated total Ca requirements between the previous authors was presumed to be related to differences in growth rates and feed efficiencies of the broilers, differences in protein and energy concentrations in the diets, and fat type and content in the experimental rations (Edwards et al., 1963).

Today, approximately 60-years later, discrepancies still exist when estimating the total Ca requirements for modern broilers; and especially when comparing the newly developed standardized ileal digestible (SID) Ca requirements for broiler chickens. Driver et al. (2005b) and Bai et al. (2022) estimated the requirement to maximize body weight gain (BWG) during the starter phase was 0.625% or 0.590% total Ca, respectively. Whereas, during the grower phase (d 19 to 42), Driver et al. (2005b) reported no significant

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effect of increasing total Ca level on BWG or bone ash. However, Rama Rao et al. (2003) suggested 0.756% total Ca was required to maximize BWG from d 1 to 35 post-hatch. In 3 of the 4 studies, approximately 1.0% total Ca was required to support Ca metabolism and optimal skeletal development (Bai et al., 2022; Driver et al., 2005b; Rama Rao et al., 2003), indicating the Ca requirement to optimize tibia ash is greater than that needed to optimize growth and feed efficiency, but the exact total Ca requirement remains elusive.

More recently, David et al. (2021) and Walk et al. (2021b) set about estimating the SID Ca requirements of fast-growing broilers from hatch to d 10. In P-adequate diets, David et al. (2021) estimated the SID Ca requirement for maximum weight gain and bone mineralization was 0.332% and 0.436% to 0.478%, respectively. Whereas Walk et al. (2021b) reported no effect of graded levels of SID Ca on BWG and tibia ash was maximized at 0.530% SID Ca. In both experiments, apparent metabolizable energy (AME), crude protein, and digestible lysine in the experimental diets were formulated to meet fast-growing broiler requirements, soybean oil was used as the supplemental fat source, and growth rates, feed intake (FI), and feed conversion ratio (FCR) were comparable between the studies. Therefore, the discrepancies in the estimated SID Ca requirements were not related to differences in growth rates or dietary composition of protein, energy, or fat sources as previously hypothesized by Edwards et al. (1960, 1963).

Other factors that might be influencing the results are the concentration of phytate and phytase in the experimental diets (Selle et al., 2009), the SID Ca coefficients used to formulate the experimental diets, which can be quite variable (Walk et al., 2021a), differences in the source, quality, and particle size of the limestone (Kim et al., 2019) used to create the graded concentrations of SID Ca, different graded levels of total Ca, and differences in broiler genetics, with Ross 308 broilers used in David et al. (2021) and Arbor Acres Plus broilers used in Walk et al. (2021b). Therefore, the current study aims to re-evaluate the previously determined SID Ca requirement for fast-growing broilers, using Ross 308 genetics, 3 different non-linear regression models including the quadratic (QP), straight-broken line (SBL) and quadratic-broken line (QBL), and a reference diet formulated using total Ca to meet or exceed nutrient recommendations, from hatch to d 14 post-hatch.

## 2. Materials and methods

### 2.1. Animal ethics

The animal protocol for this research was approved by the Ethical Committee of DSM Animal Nutrition Research Center and complied with the guidelines in the European Union council directive 2010/63/EU for animal used for experiments. This experiment was conducted in February 2021 at DSM Animal Nutrition Research Center in Village-Neuf, France.

### 2.2. Animals and housing

Three-hundred and sixty Ross 308 male broilers were obtained on the day of hatch and randomly allocated to 60 battery cages to d 14 post-hatch. There were 6 birds per cage and 12 replicate cages per dietary treatment. The cages were housed in an environmentally controlled room with a lighting program of 12L:12D for the duration of the trial. Birds were allowed ad libitum access to feed and water.

### 2.3. Diets

Five dietary treatments, based predominantly on wheat and soybean meal, were fed from day of hatch to d 14 post-hatch. Four treatments were formulated to contain graded levels of SID Ca, including 0.60%, 0.46%, 0.32%, and 0.18%. The diets were formulated to meet nutrient requirements for Ross broilers (Ross Nutrition Specifications 2019, Aviagen, Huntsville, AL), with the exception of Ca (Table 1). To mitigate any confounding effects of dietary phytate on Ca digestibility, the SID Ca diets were formulated to contain 2,000 phytase units (FYT)/kg of diet (HiPhorius, DSM Nutritional Products, Kaiseraugst, Switzerland) to contribute 0.19% non-phytate P (nPP). To ensure graded concentrations of SID Ca were achieved, a large batch of the 0.60% and 0.18% SID Ca diets were mixed and then combined at 67:33 or 33:67 ratios to create the 0.46% or 0.32% SID Ca diets, respectively. The fifth treatment was formulated using total Ca, including the same ingredients, to meet or exceed all nutrient requirements, and contained no exogenous phytase. This diet served as a reference diet to compare current nutrient

**Table 1**
Ingredient and nutrient content of the high or low standardized ileal digestible (SID) Ca diets or the reference diet (%, as-fed basis).

| Item | SID Ca, % | | Reference diet |
|---|---|---|---|
| | 0.60 | 0.18 | |
| Ingredient | | | |
| Wheat | 65.37 | 68.60 | 60.47 |
| Soybean meal | 24.90 | 24.09 | 27.46 |
| Rapeseed meal (solvent extracted) | 3.50 | 3.50 | 3.50 |
| Soy oil | 2.14 | 1.25 | 4.41 |
| Salt | 0.43 | 0.43 | 0.43 |
| Limestone[1] | 1.64 | 0.10 | 0.55 |
| Dicalcium phosphate | 0.72 | 0.70 | 1.88 |
| Lysine HCl | 0.32 | 0.34 | 0.32 |
| DL-Methionine | 0.29 | 0.29 | 0.30 |
| Threonine | 0.17 | 0.18 | 0.18 |
| Premix (UH19733002)[2] | 0.40 | 0.40 | 0.40 |
| Protease[3] | 0.0050 | 0.0050 | 0.00 |
| Phytase[4] | 0.0144 | 0.0144 | 0.00 |
| Carbohydrase[5] | 0.0075 | 0.0075 | 0.00 |
| Titanium dioxide | 0.10 | 0.10 | 0.10 |
| Formulated nutrient content | | | |
| Crude protein | 21.50 | 21.50 | 21.50 |
| AME, kcal/kg | 3050.00 | 3050.00 | 3050.00 |
| DM | 88.30 | 88.01 | 88.57 |
| Total Ca | 0.98 | 0.37 | 0.90 |
| SID Ca | 0.60 | 0.18 | 0.45 |
| Total P | 0.52 | 0.52 | 0.74 |
| Available P | 0.45 | 0.45 | 0.45 |
| Digestible P | 0.40 | 0.40 | 0.43 |
| Non-phytate P | 0.46 | 0.46 | 0.49 |
| Phytate P | 0.25 | 0.24 | 0.25 |
| Digestible methionine + cystine | 0.91 | 0.91 | 0.91 |
| Digestible lysine | 1.22 | 1.22 | 1.22 |
| Digestible threonine | 0.83 | 0.83 | 0.83 |
| Sodium | 0.18 | 0.18 | 0.18 |
| Chloride | 0.40 | 0.40 | 0.41 |

[1] The limestone used in the study had a particle size (GMD) of 331 μm, a total Ca of 38.88% and an in vitro solubility (Kim et al., 2019) of 95.84% and 97.68% at 15 and 30 min, respectively. The estimated digestibility coefficient using the glycine buffer equation for phytase from Kim et al. (2019) was 0.698.

[2] Vitamin and mineral premix provided (per kilogram of diet): vitamin A 10,000 IU; vitamin $D_3$ 3,000 IU; vitamin E 40 mg; vitamin $K_3$ 3 mg; vitamin $B_1$ 2.5 mg; vitamin $B_2$ 8 mg; vitamin $B_6$ 5 mg; vitamin $B_{12}$ 0.025 mg; biotin 0.15 mg; folic acid 1.5 mg; niacinamide 50 mg; D-pantothenic acid 12 mg; Fe (as $FeSO_4$) 60 mg; Cu (as $CuSO_4$) 15 mg; Mn (as MnO) 80 mg; Zn (as ZnO) 54 mg; I (as $Ca(IO_3)_2$) 1.2 mg; Se (as $Na_2SeO_3$) 0.297 mg; calcium as calcium carbonate (carrier) 26.4 mg.

[3] ProAct 360 (DSM Nutritional Products, Kaiseraugst, Switzerland) with an analyzed activity of 785,470 New Feed Protease units (NFP)/g, contributed 0.67% crude protein, 0.04% methionine + cysteine, 0.05% lysine, and 0.05% threonine.

[4] HiPhorius (DSM Nutritional Products, Kaiseraugst, Switzerland) with an analyzed activity of 13,850 phytase units (FYT)/g, contributed 0.19% non-phytate P or 0.15% digestible P.

[5] Ronozyme WX 2000 CT (DSM Nutritional Products, Kaiseraugst, Switzerland) with an analyzed activity of 2,590 fungal xylanase units (FXU)/g and contributed 120 kcal/kg metabolizable energy.

requirements with the SID Ca diets and adds to validate the estimated SID Ca requirements. All 5 experimental diets were isonitrogenous and isocaloric. Diets were pelleted at 70 °C and fed in crumble form. Titanium dioxide was added to the experimental diets at 0.10% as an inert marker for determination of nutrient digestibility and retention.

In the diets formulated to achieve the graded levels of SID Ca, the apparent ileal digestibility (AID) coefficient of Ca in limestone was estimated at 0.698 (with phytase) by using in vitro solubility measured at 15 (95.8%) and 30 (97.7%) min and mean particle size (331 μm) according to the methods of Kim et al. (2019). The ingredients (wheat, soybean meal, rapeseed meal, limestone, dicalcium phosphate, and the vitamin and mineral premix) were analyzed for nutrient composition according to the standard methods (VDLUFA, 1976), including total Ca and P. The analyzed total Ca concentrations and previously estimated SID Ca coefficients (Walk et al., 2021a) were used to formulate the experimental diets to achieve graded levels of SID Ca. Nutrient requirements for AME and digestible amino acids were achieved using ingredient specifications from the Premier Nutrition Atlas (Premier Nutrition Ltd, Rugeley, UK). Digestible P coefficients for the ingredients were estimated from CVB (2021), available P concentrations were obtained from Rostango et al. (2017), and phytate P concentrations were obtained from a combination of results from Aureli et al. (2017), Rostango et al. (2017), and Tahir et al. (2012).

Xylanase and protease activities in the diets were analyzed using methods based on dye-labelled substrates (Azo-Xylan and Suc-Ala-Ala-Pro-Phe-pNA, respectively). Phytase activity was measured by Method PHY-102/06E DSM. 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.

### 2.4. Data and sample collection

At placement (d 0) and d 14, all birds were weighed by cage to determine mean BW and calculate mean BWG. Feed added and feed left over were weighed at d 0 and d 14 to calculate FI. Body weight gain and FI were used to calculate 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 pen.

On d 14, after weighing, all remaining birds per cage were euthanized by cervical dislocation. Ileal digesta (defined as the Meckel's diverticulum to 40 mm proximal to the ileocecal junction) was collected on ice from the distal two-thirds of tract by squeezing, pooled within pen, and immediately frozen. Excreta were collected daily from d 12 to 14, pooled and homogenized within cage, and frozen until further analysis.

### 2.5. Apparent nutrient utilization

Digesta and excreta were freeze dried (Christ Epsilon 2-10D LSC, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) to a constant weight. Diets, digesta, and excreta were ground using a hammer mill (Fritsch Pulverisette 14, Fritsch GmbH – Idar-Oberstein, Germany) to pass a 0.5-mm screen and then analyzed for Ca, P, and Ti using inductively coupled plasma-optical emission spectrometry (ICP-OES, 5100 Dual View, Agilent, Santa Clara, California) after sulfuric acid mineralization (based on method 985.01; AOAC International, 2006). Calcium, P, and Ti were then used to determine AID and apparent total tract retention (ATTR) using the Ti marker ratios in the diet and digesta or excreta (Ravindran et al., 1999).

### 2.6. Tibia ash

On d 14, after weighing, left tibias were obtained from 3 of the birds per cage that were euthanized for ileal digesta collection. Tibias were pooled within cage and stored frozen. Tibias were stripped of adhering tissues, dried at 105 °C for 24 h (Binder incubator ED400, Binder GmbH, Tuttlingen, Germany), and then ashed at 550 °C for 48 h in a muffle furnace (Nabertherm B170, Nabertherm GmbH, Lilienthal, Germany) for determination of tibia ash percent and weight.

### 2.7. Statistical analysis

Data were subjected to an analysis of variance using JMP Pro v16.0 (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 ATTR 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 at 100% (or the maximum value) of the requirement using various methods of non-linear regression in JMP Pro v16.0 (SAS Institute, Cary NC). The models included polynomial (linear or quadratic), SBL, or QBL regression models. Significance was accepted at $P < 0.05$ for all statistical evaluations.

## 3. Results

### 3.1. Experimental diets

Enzyme activities recovered in the experimental diets are presented in Table 2. Phytase (120%) and xylanase (83%) recoveries were as expected when considering product overages and analytical variation. Protease recoveries were greater than expected (168%), most likely due to a higher activity in the product than expected. In general, analyzed total Ca, total P, and crude protein agreed with formulated values (Table 2). Graded concentrations of total Ca were achieved at each concentration of SID Ca. The total P concentration was also different between the SID Ca diets and the

**Table 2**
Analyzed nutrient content of the experimental diets.[1]

| Item | Standardized ileal digestible calcium, % | | | | Reference diet |
|---|---|---|---|---|---|
| | 0.60 | 0.46 | 0.32 | 0.18 | |
| Analyzed nutrients, % | | | | | |
| Dry matter | 88.94 | 89.25 | 89.13 | 88.82 | 89.19 |
| Crude protein | 22.59 | 22.47 | 22.38 | 22.19 | 23.69 |
| Total calcium | 1.04 | 0.83 | 0.58 | 0.39 | 0.98 |
| Total phosphorus | 0.55 | 0.55 | 0.56 | 0.56 | 0.75 |
| Enzyme activity | | | | | |
| Phytase, FYT/kg | 2,546 | 2,196 | 2,258 | 2,611 | 382 |
| Xylanase, FXU/kg | 129 | 108 | 133 | 124 | LOD |
| Protease, NFP/kg | 40,080 | 52,190 | 53,160 | 55,710 | LOQ |

FYT = phytase units; FXU = fungal xylanase units; NFP = New Feed Protease units; LOD = limits of detection; LOQ = limits of quantification.
[1] Diets were analyzed in duplicate.

reference diet confirming the use of a P matrix for phytase in the SID Ca diets.

### 3.2. Growth performance

There was a significant impact of diet on FI, BWG, and mortality corrected feed conversion ratio (mFCR) from hatch to d 14 post-hatch (Table 3). Birds fed the reference diet ate less ($P < 0.05$) but had a similar BWG or mFCR to that of birds fed 0.60%, 0.46%, or 0.32% SID Ca. Decreasing SID Ca content in the diet from 0.60% to 0.18% linearly ($P < 0.05$) reduced FI. Body weight gain was lowest in birds fed 0.18% SID Ca, increased and similar between birds fed 0.32% or 0.46% SID Ca, and highest in birds fed 0.60% SID Ca, resulting in a quadratic ($P < 0.05$) effect of SID Ca on BWG. Mortality corrected FCR was higher in birds fed 0.18% or 0.32% SID Ca and decreased to a similar level in birds fed 0.46% to 0.60% SID Ca, resulting in a quadratic ($P < 0.05$) effect of SID Ca on mFCR.

### 3.3. Tibia ash

Tibia ash percentage (quadratic, $P < 0.05$) and ash weight (quadratic, $P < 0.50$) were greatest in birds fed 0.60% or 0.46% SID Ca and then decreased as the SID Ca content in the diet decreased to 0.32% and 0.18% (Table 4). Birds fed 0.32% and 0.18% SID Ca had a lower ($P < 0.05$) tibia ash percentage or ash weight compared with birds fed the reference diet.

### 3.4. Apparent nutrient utilization

Apparent ileal dry matter, Ca and P digestibility were influenced by diet, whereas there was no effect of diet on apparent ileal N digestibility (Table 5). Increasing the SID Ca content in the diet linearly ($P < 0.05$) increased ileal DM digestibility and this was comparable to birds fed the reference diet, except birds fed 0.18% SID Ca (lower, $P < 0.05$). Apparent ileal Ca digestibility was similar between birds fed 0.60% and 0.46% SID Ca and those fed the reference diet. However, as the SID Ca content in the diet decreased to 0.32% or 0.18%, the AID of Ca significantly increased (quadratic, $P < 0.05$) and this was greater ($P < 0.05$) than in birds fed the reference diet. Apparent ileal P digestibility was similar between birds fed 0.60% and 0.46% SID Ca and increased as the SID Ca content in the diet decreased (quadratic, $P < 0.05$) to 0.18% SID Ca. Formulating diets using SID Ca and including high doses of exogenous phytase increased ($P < 0.05$) the AID of P compared with birds fed the reference diet, regardless of the SID Ca content. Apparent ileal digested Ca linearly ($P < 0.05$) decreased as the SID Ca content in the diet decreased. This resulted in a good agreement ($r = 0.99$) between the formulated and expected SID Ca concentrations in the diets. However, there were slight differences between measured and formulated values above and below the determined requirement. Therefore, the measured apparent ileal digested Ca was also used to estimate the requirements and were described as the AID Ca requirements. Apparent ileal digested P improved quadratically ($P < 0.05$) as SID Ca content in the diet decreased from 0.60% to 0.18%, with the greatest increase in digested P between birds fed 0.46% and 0.32% SID Ca.

Apparent dry matter, nitrogen, Ca, and P retention were influenced by diet (Table 6). Apparent N and P retention linearly ($P < 0.05$) increased as the SID Ca content of the diet increased from 0.18% to 0.60%. Apparent P retention was greater ($P < 0.05$) in all birds fed SID Ca compared with birds fed the reference diet. However, apparent nitrogen retention was greater ($P < 0.05$) in birds fed the reference diet compared with birds fed SID Ca at 0.18% or 0.32%. Finally, apparent Ca retention was lowest in birds fed 0.60% or 0.46% SID Ca and increased (quadratic, $P < 0.05$) as the SID Ca content of the diet decreased from 0.32% to 0.18% and this was greater ($P < 0.05$) than in birds fed the reference diet.

### 3.5. Estimated digestible calcium requirements

Growth performance (BWG and mFCR), tibia ash percent and weight, and Ca and P utilization were used to estimate the SID Ca requirement of fast-growing broilers from hatch to d 14. Three different non-linear models were used to determine the SID Ca requirement (Table 7). In general, the QBL model resulted in the lowest estimated SID Ca requirement (0.398%) followed by the SBL (0.480%) and then the QP model (0.534%). This was partially true when the requirements were estimated using the measured AID of Ca, with the QBL and SBL estimating similar requirements at 0.436% and 0.430%, respectively, and the requirements estimated higher at 0.495% when the QP was used. Growth performance was less sensitive to the SID Ca content in the diets, and this resulted in

**Table 3**
Growth performance of broilers fed graded concentrations of standardized ileal digestible (SID) Ca from hatch to d 14 post-hatch.[1]

| Item | Feed intake, g | BWG, g | mFCR,[2] g:g | Liveability, % |
|---|---|---|---|---|
| SID Ca, % | | | | |
| 0.60 | 603** | 495 | 1.222 | 98.6 |
| 0.46 | 592* | 486 | 1.219 | 98.6 |
| 0.32 | 604** | 486 | 1.244** | 100.0 |
| 0.18 | 568 | 438* | 1.283*** | 98.6 |
| Reference diet[3] | 559 | 469 | 1.193 | 100.0 |
| SEM | 9.1 | 8.5 | 0.0098 | 1.10 |
| *P*-value | | | | |
| Diet[4] | 0.0018 | 0.0002 | <0.0001 | 0.7515 |
| Linear SID Ca | 0.0292 | 0.0001 | <0.0001 | – |
| Quadratic SID Ca | 0.2071 | 0.0283 | 0.0405 | – |

BWG = body weight gain; mFCR = mortality corrected feed conversion ratio.
[1] Data are least square means of 6 birds per pen and 12 replicate pens per treatment.
[2] Mortality corrected feed conversion ratio.
[3] The reference diet was formulated to meet or exceed nutrient requirements for fast growing broilers, using 0.90% total calcium and 0.45% available P, 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 4**
Tibia ash of broilers fed graded concentrations of standardized ileal digestible (SID) Ca from hatch to d 14 post-hatch.[1]

| Item | Tibia ash, % | Tibia ash weight, g/bone |
|---|---|---|
| SID Ca, % | | |
| 0.60 | 55.43 | 0.534 |
| 0.46 | 54.86 | 0.504 |
| 0.32 | 54.17** | 0.485 |
| 0.18 | 51.32*** | 0.383*** |
| Reference diet[2] | 55.55 | 0.493 |
| SEM | 0.236 | 0.0090 |
| *P*-value | | |
| Diet[3] | <0.0001 | <0.0001 |
| Linear SID Ca | <0.0001 | <0.0001 |
| Quadratic SID Ca | <0.0001 | 0.0050 |

[1] Data are least square means of 3 birds per pen and 12 replicate pens per treatment.
[2] The reference diet was formulated to meet or exceed nutrient requirements for fast growing broilers, using 0.90% total calcium and 0.45% available P, without exogenous enzymes.
[3] 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). **$P < 0.01$, ***$P < 0.0001$.

**Table 5**
Apparent ileal nutrient digestibility of broilers fed graded concentrations of standardized ileal digestible (SID) Ca from hatch to d 14 post-hatch.[1]

| Item | Apparent ileal digestibility | | | | Apparent ileal digested | |
|---|---|---|---|---|---|---|
| | Dry matter, % | Nitrogen, % | Calcium, % | Phosphorus, % | Calcium, % | Phosphorus, % |
| SID Ca, % | | | | | | |
| 0.60 | 60.07 | 80.01 | 44.52 | 60.03*** | 0.528* | 0.371 |
| 0.46 | 57.80 | 78.75 | 44.82 | 61.59*** | 0.417 | 0.381* |
| 0.32 | 58.57 | 79.42 | 53.47** | 69.36*** | 0.346** | 0.435*** |
| 0.18 | 52.57** | 77.11 | 65.41*** | 76.91*** | 0.274*** | 0.484*** |
| Reference diet[2] | 60.84 | 77.96 | 40.97 | 42.36 | 0.449 | 0.358 |
| SEM | 1.695 | 0.827 | 1.897 | 0.996 | 0.0225 | 0.0061 |
| *P*-value | | | | | | |
| Diet[3] | 0.0055 | 0.1452 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Linear SID Ca | 0.0034 | – | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Quadratic SID Ca | 0.2107 | – | 0.0014 | 0.0010 | 0.2944 | 0.0017 |

[1] Data are least square means of all birds per pen and 12 replicate pens per treatment.
[2] The reference diet was formulated to meet or exceed nutrient requirements for fast growing broilers, using 0.90% total calcium and 0.45% available P, without exogenous enzymes.
[3] 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 6**
Apparent excreta nutrient retention of broilers fed graded concentrations of standardized ileal digestible (SID) Ca from hatch to d 14 post-hatch.[1]

| Item | Apparent excreta retention | | | |
|---|---|---|---|---|
| | Dry matter, % | Nitrogen, % | Calcium, % | Phosphorus, % |
| SID Ca, % | | | | |
| 0.60 | 65.38 | 65.06 | 49.86 | 61.66*** |
| 0.46 | 64.85 | 63.38 | 55.62** | 59.61*** |
| 0.32 | 64.24 | 62.65* | 63.02*** | 59.10*** |
| 0.18 | 60.66** | 61.80** | 74.52*** | 54.58*** |
| Reference diet[2] | 65.22 | 65.23 | 50.61 | 44.53 |
| SEM | 0.731 | 0.623 | 0.976 | 0.698 |
| *P*-value | | | | |
| Diet[3] | <0.0001 | 0.0011 | <0.0001 | <0.0001 |
| Linear SID Ca | <0.0001 | 0.0006 | <0.0001 | <0.0001 |
| Quadratic SID Ca | 0.0316 | 0.5195 | 0.0066 | 0.0986 |

[1] Data are least square means from 12 replicate pens per treatment.
[2] The reference diet was formulated to meet or exceed nutrient requirements for fast growing broilers, using 0.90% total calcium and 0.45% available P, without exogenous enzymes.
[3] 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). *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$.

greater model variation ($R^2 = 0.23$ to 0.38) compared with tibia ash ($R^2 = 0.56$ to 0.65) or Ca and P utilization ($R^2 = 0.43$ to 0.84; Table 7). However, the estimated SID Ca or AID Ca requirements for BWG (0.42% to 0.52%) or mFCR (0.39% to 0.52%) were within those estimated using tibia ash percent (0.33% to 0.54%), tibia ash weight (0.32% to 0.58%), AID Ca (0.40% to 0.57%), AID P (0.38% to >0.60%), Ca retention (0.36% to >0.60%) or P retention (0.45% to >0.60%). Taking the average of the estimates from all response parameters and the three experimental models, the formulated SID Ca or measured AID Ca requirement of fast-growing broilers from hatch to d 14 post-hatch was estimated at 0.471% or 0.454%, respectively. This is equivalent to a formulated SID Ca or measured AID Ca to nPP ratio of 1.024 or 0.987, respectively, or a measured apparent ileal digested P ratio of 1.198 or 1.185 for formulated SID Ca or measured AID Ca, respectively.

## 4. Discussion

Calcium and P are the most abundant minerals in the body and are predominantly found in the skeleton. Dietary imbalances or deficiencies of Ca or P and excess dietary Ca can lead to reduced growth performance and nutrient digestion (particularly P and amino acids), as well as poor feed efficiency and bone mineralization and may even lead to death in severe cases. Understanding the amount of dietary Ca and P that is digested, absorbed, and used by the animal is vitally important for bird health and welfare and promotes efficient use of inorganic P. Formulating broiler diets to meet digestible, rather than total nutrient requirements has resulted in improvements in BWG, feed efficiency, and reduced nutrient excretion. For example, Rostango et al. (1995) found that birds fed diets based on digestible amino acids were more efficient and gained more compared with birds fed diets based on total amino acids. Interestingly, at that time, the digestible amino acid diet was more expensive; but because birds were more efficient and performed better, feed costs per kilogram of BW were lower in birds fed the digestible amino acid diet (Rostango et al., 1995). Similarly, formulating diets closer to the bird's requirement for P can reduce the amount of P excreted by the bird by 30% to 40% (Applegate and Angel, 2008).

The long-term objective of this work is to optimize the inclusion of Ca in broiler diets, thereby improving growth performance, feed efficiency, and P and amino acid utilization through the implementation of a SID Ca formulation system. The specific objective of this study was to estimate the SID Ca requirement of fast-growing broilers from hatch to d 14 post-hatch using QP, SBL, and QBL models, to provide additional and comparative information on the SID Ca requirement of broilers. The models were selected based on previously published methods to estimate nutrient requirements of broilers. Each non-linear model has benefits and limitations and these should be considered when setting nutrient requirements. For example, the QP model estimates the requirement at the concentration of nutrient that results in the maximum response. There is a single maximum, not a plateau, and anything below or above the maximum is considered lower predicted performance or nutrient utilization levels (Pesti et al., 2009). In this regard, the QP model may be useful to estimate deficiencies or toxicities, but generally results in a greater estimated requirement compared with the SBL or QBL (Pesti et al., 2009; Walk et al., 2022). When using the QP, it may be possible to estimate the requirement at a value other than the maximum, such as 90% to 99% of the maximum (Pesti et al., 2009), to account for the higher estimated requirements compared with other non-linear models. The SBL model presumes that the response to a nutrient dose is linear until a plateau and no further changes in the requirement are noted (Robbins et al., 2006). In this case, the linear assumption may

**Table 7**
Comparison of the prediction models and the estimated digestible Ca requirement of broilers from hatch to d 14 post-hatch.

| Parameter | Model | $R^2$ | RMSE | *P*-value | Estimated requirement | |
|---|---|---|---|---|---|---|
| | | | | | SID Ca, %[1] | AID Ca, %[2] |
| Body weight gain, g | Quadratic[3] | 0.31 | 105.0 | <0.05 | 0.475 | 0.464 |
| | Straight-broken line | 0.23 | 37.4 | <0.05 | 0.517 | 0.418 |
| | Quadratic-broken line | 0.24 | 37.2 | <0.05 | 0.472 | 0.481 |
| mFCR[4], g:g | Quadratic[3] | 0.38 | 0.10 | <0.05 | 0.515 | 0.470 |
| | Straight-broken line | 0.33 | 0.04 | <0.05 | 0.394 | 0.407 |
| | Quadratic-broken line | 0.34 | 0.04 | <0.05 | 0.404 | 0.401 |
| Tibia ash, % | Quadratic[3] | 0.59 | 1.43 | <0.05 | 0.540 | 0.479 |
| | Straight-broken line | 0.56 | 1.50 | <0.05 | 0.470 | 0.423 |
| | Quadratic-broken line | 0.61 | 1.42 | <0.05 | 0.327 | 0.426 |
| Tibia ash weight, g/bone | Quadratic[3] | 0.62 | 0.14 | <0.05 | 0.575 | 0.493 |
| | Straight-broken line | 0.61 | 0.14 | <0.05 | 0.400 | 0.422 |
| | Quadratic-broken line | 0.65 | 0.13 | <0.05 | 0.320 | 0.479 |
| Apparent ileal Ca digestibility, % | Quadratic[3] | 0.60 | 7.49 | <0.05 | 0.565 | 0.481 |
| | Straight-broken line | 0.55 | 7.92 | <0.05 | 0.570 | 0.398 |
| | Quadratic-broken line | 0.60 | 7.60 | <0.05 | 0.412 | 0.433 |
| Apparent Ca retention, % | Quadratic[3] | 0.84 | 4.14 | <0.05 | >0.60 | >0.528 |
| | Straight-broken line | 0.84 | 4.22 | <0.05 | 0.535 | 0.516 |
| | Quadratic-broken line | 0.84 | 4.22 | <0.05 | 0.360 | 0.432 |
| Apparent ileal P digestibility, % | Quadratic[3] | 0.70 | 4.80 | <0.05 | >0.60 | >0.528 |
| | Straight-broken line | 0.68 | 5.00 | <0.05 | 0.400 | 0.400 |
| | Quadratic-broken line | 0.70 | 4.87 | <0.05 | 0.383 | 0.385 |
| Apparent P retention, % | Quadratic[3] | 0.45 | 2.89 | <0.05 | >0.60 | 0.519 |
| | Straight-broken line | 0.43 | 2.92 | <0.05 | 0.550 | 0.456 |
| | Quadratic-broken line | 0.43 | 2.92 | <0.05 | 0.509 | 0.449 |
| Mean estimated SID Ca requirement, % | Quadratic[3] | 0.56 | 15.70 | – | 0.534 | 0.495 |
| | Straight-broken line | 0.53 | 7.39 | – | 0.480 | 0.430 |
| | Quadratic-broken line | 0.55 | 7.30 | – | 0.398 | 0.436 |

mFCR = mortality corrected feed conversion ratio.
[1] The estimated standardized ileal digestible Ca requirement using formulated concentrations. The $R^2$, root mean square error (RMSE), and *P*-value presented are based on the SID Ca values.
[2] The estimated apparent ileal digestible Ca requirement using measured concentrations.
[3] Estimated using the maximum response.
[4] Mortality corrected feed conversion ratio.

simplify the impact of dietary Ca level on performance or nutrient utilization by assuming the rate of change is the same with each change in nutrient concentration in the diet. In the current study, the significant quadratic effects of dietary SID Ca on growth performance, mFCR, tibia ash, ileal digestibility, and retention of Ca and P indicate the response of the bird differs depending on the SID Ca concentration in the diet and a SBL might simplify this effect. In addition, the SBL assumes additional supplementation of the nutrient at concentrations above the break point or plateau has no negative impacts on growth performance or nutrient utilization. This may be the case for some nutrients, but high concentrations of total dietary Ca have been shown to reduce growth performance (Amerah et al., 2014; Bai et al., 2022; Simco and Stephenson, 1961) and P and amino acid digestibility (Amerah et al., 2014) of broilers, especially when dietary P is limiting. Therefore, use of the SBL may over-simplify the effect of dietary Ca on broiler performance, efficiency, and nutrient utilization. Finally, the QBL model was considered as a method to estimate both the curvilinear (Robbins et al., 2006) and potential toxic (Pesti et al., 2009) effects of dietary SID Ca concentrations on growth performance, tibia ash, and nutrient utilization. In the current study, the QBL model resulted in the lowest estimated SID Ca requirements, especially compared with the QP model. This was also true for the SID Ca requirements estimated by David et al. (2021) for tibia ash concentration (0.451%). Walk et al. (2022) also found the QBL model generally estimated the SID Ca requirements at lower dietary concentrations when compared with the SBL and QP. Whereas Robbins et al. (2006) reported the QBL provided a greater estimated isoleucine requirement for growing pigs when compared with the SBL. Unfortunately, and as found with other nutrient requirement studies, choosing the best model to estimate the requirement is not simple. It may be possible to select the best regression model using the coefficient of determination statistics, such as $R^2$, adjusted $R^2$, root mean square error, and models with lower Akaike information criterion (AIC) or Bayesian information criterion (BIC) coefficients, assuming all factors are significant (Pesti et al., 2009). Another option to select the optimum requirement could be to use practical considerations, such as feed cost savings or cost per bird. In this case, the best feeding level is the nutrient concentration that maximizes profits (Pesti et al., 2009). The economic model has been considered for digestible amino acids (Kidd and Tillman, 2016; Rostango et al., 1995) and may be something to consider when utilizing the SID Ca requirement recommendations in practice.

When using the mean of the parameters and non-linear models in the current study, the estimated formulated SID Ca or measured AID Ca requirement was 0.471% or 0.454%, respectively, for broilers from hatch to d 14. This is equivalent to a SID Ca to nPP ratio of 1.024 or 0.987, respectively, or a measured apparent ileal digested P ratio of 1.198 or 1.185 for formulated SID Ca or measured AID Ca, respectively. The SID or AID Ca to digested P ratio is similar to that reported by Angel (2017; 1.15) but higher than that reported by David et al. (2021; 0.66 to 0.96) in broilers from hatch to d 10. The estimated SID Ca requirements in the current study were slightly lower than those obtained by Walk et al. (2021b) and similar to or higher than those obtained by David et al. (2021) for optimal tibia ash (0.478%) and BWG (0.332%), respectively, when comparing data from the QP model and male broilers from hatch to d 10.

Differences in the findings between the studies may be related to differences in the SID Ca coefficients of the ingredients, the use of high doses of phytase in the current study, differences in the concentrations of total Ca in the diets, or differences in the P formulation systems, particularly the digestible P (dgP) coefficients. Upon comparison of the previous work by David et al. (2021) and the current study, we found the SID Ca coefficients were similar between the studies and the analyzed total Ca concentrations were wider in the current study but within the range of that tested by David et al. (2021; 1.04% to 0.39% and 1.16% to 0.69%, respectively). In fact, when the ingredient composition from David et al. (2021) was input into the feed formulation software used to formulate the diets for the current experiment, the SID Ca values only differed by 0.01 to 0.03 percentage units. The total P and nPP concentrations were also very similar between the 2 studies, whereas there were large differences in the dgP coefficients used between the different experimental diets. Therefore, the differences in the estimated requirements between the studies were most likely not related to differences in the SID Ca coefficients, total P, or nPP, but rather differences between the formulated dgP concentrations in the experimental diets. For example, when comparing the formulation systems and using the diet and ingredient composition of the treatments formulated to contain 0.40% SID P from David et al. (2021), total P was listed as 0.53% in the paper and 0.56% in the current study formulation software. However, dgP was only calculated at 0.29% in the current formulation software and this is nearly 30% below the expected 0.40% SID P. The dgP coefficients used in the David et al. (2021) study for corn, soybean meal, and inorganic P were based on data from the same lab and the coefficients used were 0.70, 0.75, and 0.79, respectively. Whereas, the dgP coefficients in the current study are from CVB (2021) values, which estimate the P digestibility of corn, soybean meal, and inorganic phosphates at much lower coefficients of 0.30, 0.42, and 0.78, respectively. In addition, based on CVB (2021), dgP coefficients are determined from fecal samples and not adjusted for endogenous P losses, which may also be a big factor influencing the differences in the coefficients. However, when using standardized experimental methods and the same soybean meal, previous authors reported the dgP concentration of soybean meal ranged from 19% to 51% (Rodehutscord et al., 2017). This difference in dgP concentration in the diet formulations, plus the inclusion of high doses of phytase in the diets of the current study may have resulted in the big differences in the estimated SID Ca requirements between the different labs.

The impact of dietary P concentration on the Ca requirement is well known. For example, in 1961, Simco and Stephenson found when a lower level of total P was fed (0.42%), feeding excessive total dietary Ca (1.0%) had a greater detrimental effect on growth performance compared with feeding lower concentrations of total Ca. Similarly, widening the Ca to total P ratio in broiler diets containing phytase had detrimental effects on growth performance (Driver et al., 2005a). David et al. (2021) reported the SID Ca requirement to optimize BWG or tibia ash was different depending on the dgP concentration in the diet. To avoid differences between the digestibility systems in the different studies and its impact on the interpretation of the requirements, the authors of the current study chose to present the estimated SID Ca requirements as a mean of 3 regression models, using various response variables, and as a ratio to the nPP concentration in the experimental diets. Non-phytate P is useful because it can be analyzed in the diets using standardized assays of total P and phytate P and then subtracting phytate P from the total P. Furthermore, because the apparent ileal digested P was also measured in the current study, it was possible to determine the apparent ileal digested P at the estimated SID Ca (0.471%) or measured AID Ca (0.454%) requirements and therefore present the mean digestible Ca to dgP ratio from the results. We hope using nPP and/or measured apparent ileal digested P at the estimated SID Ca requirement will aid in establishing aligned digestible Ca requirements and recommendations in the future.

## 5. Conclusions

In conclusion, the formulated SID Ca and measured AID Ca requirement of fast-growing broilers from hatch to d 14 was estimated at 0.534%, 0.480%, or 0.398%, and 0.495%, 0.430%, or 0.436% when QP, SBL, or QBL regression models were employed, respectively. Taking the average of the estimates from all response parameters and the 3 non-linear models, the formulated SID Ca requirement of fast-growing broilers from hatch to d 14 post-hatch was estimated at 0.471% and this is equivalent to a SID Ca to nPP ratio of 1.024 or a SID Ca to measured apparent ileal digested P ratio of 1.198. When considering the measured apparent ileal digested Ca, the Ca requirement was estimated at 0.454% and equivalent to a nPP or measured apparent ileal digested P ratio of 0.987 or 1.185, respectively. These results support the use of digestible Ca in feed formulation for broilers from hatch to d 14. They are validated by previously published data as well as the use of the nutrient adequate reference diet. The estimated SID Ca requirements in the current study were similar to previously published data from the same lab, but higher than those previously reported by David et al. (2021) and this may be due to differences in the dgP concentration between the studies. Therefore, the use of nPP or measured apparent ileal digested P may be useful to include in studies in the future to compare SID Ca requirements at measurable and comparable levels of dietary P.

## Author contributions

**Carrie L. Walk:** Conceptualization, Methodology, Project administration, Formal analysis, Writing – original draft, Writing – reviewing and editing, Supervision. **Raffaella Aureli:** Investigation, Project administration, Formal analysis, Supervision. **Pauline Jenn:** Investigation, Formal analysis, Resources.

## Declaration of competing interest

The authors are employees of DSM and have no conflicting personal, financial, or professional interests in this work.

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