Requirement of digestible calcium at different dietary concentrations of digestible phosphorus for broiler chickens 3. Broiler finishers (d 25 to 35 post-hatch)

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ABSTRACT An experiment was conducted to determine the digestible calcium (**Ca**) and digestible phosphorous (**P**) requirements of 25 to 35-day-old broiler chickens. Fifteen corn-soybean meal-based diets containing 2.0, 2.5, 3.0, 3.5, and 4.0 g/kg standardized ileal digestible (**SID**) Ca and 2.5, 3.5, and 4.5 g/kg SID P were fed to broilers from d 25 to 35 post-hatch. Each experimental diet was randomly allocated to 6 replicate cages (8 birds per cage). Body weight and feed intake were recorded, and the feed conversion ratio was calculated. On d 35, birds were euthanized to collect the ileal digesta, tibia, and carcass for the determination of ileal Ca, and P digestibility, concentrations of ash, Ca, and P in tibia and the retention of Ca and P in the carcass. Titanium dioxide (5.0 g/kg) was included in all diets as an indigestible indicator for the ileal digestibility measurement. Feed intake and total excreta output were measured during the last 4 d of the experimental period for the measurement of apparent total tract retention of Ca and P. Fixed effects of the experiment were dietary concentrations of SID Ca and SID P and their interaction. If the interaction or main effects were significant ($P < 0.05$), the parameter estimates for second-order response surface model (**RSM**) were determined using General Linear Model procedure of SAS. The maximum response was not predicted for most of the parameters (including growth performance and tibia) as the Ca effect was linear which indicated that the highest level of Ca employed in the study may have not been high enough. The requirement of dietary SID Ca for maximization of these parameters, therefore, depends on the dietary SID P concentration when the dietary SID Ca is within 2.0 to 4.0 g/kg. However, based on the factorial analysis, the highest weight gain was observed at 3.5 g/kg SID P and 3.5 g/kg SID Ca concentrations. Tibia ash was higher in birds fed 4.5 g/kg SID P and was unaffected by dietary SID Ca concentrations. However, based on overall findings, a combination of 3.5 g/kg SID P and 3.0−3.5 g/kg SID Ca may be recommended for the optimum tibia ash. The recommended SID Ca requirements (at 3.5 g/kg SID P) for weight gain (3.5 g/kg or 6.4 g/kg total Ca) and tibia ash (3.0−3.5 g/kg or 5.5−6.4 g/kg total Ca) are lower than the current Ca recommendations (7.8 g/kg total Ca equivalent to 4.25 g/kg SID Ca; Ross, 2019) for broiler finishers, suggesting possible excess of Ca in diets formulated based on the current recommendation.

Key words: broiler finishers, digestible calcium, digestible phosphorous, weight gain, tibia ash

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INTRODUCTION

Calcium (**Ca**) and phosphorus (**P**) are essential minerals for the growth and skeletal development in broiler chickens. According to Ross 308 (2019) nutrient recommendations, the requirements of total Ca and available P (**aP**) for broilers vary between 7.2−9.6 and 3.6−4.8 g/kg, respectively, depending on the growth phase. Currently, there is a legitimate scenario for formulations based on digestible P and digestible Ca (WPSA, 2013). Recent interest in shifting feed formulations to digestible Ca (Anwar et al., 2015; 2016a,b,c; 2017; 2018; David et al., 2019; 2020; 2021a,b,c) and digestible P (Mutucumarana et al., 2014b,c; 2015a,b; Mutucumarana and Ravindran, 2016) has provided impetus for studies to estimate the requirements of digestible Ca and digestible P for broilers. Two studies have been conducted recently in our laboratory to determine the standardized ileal digestible (**SID**) Ca and SID P requirements for broiler starters (d 1−10 post-hatch; David et al., 2021c) and broiler growers (d 11−24 post-hatch; David et al., 2022) for growth, bone mineralization, and mineral utilization. The present study, third in this series, focuses on the requirements of

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SID Ca and SID P for the maximization of growth performance, bone mineralization, and Ca and P retention in broiler finishers during d 25 to 35 post-hatch.

MATERIALS AND METHODS

The experiment was conducted according to the New Zealand Revised Code of Ethical Conduct for the use of live animals for research, testing and teaching, and approved by the Massey University Animal Ethics Committee.

Experimental Diets

The ingredients (corn, soybean meal, limestone, dicalcium phosphate, and monosodium phosphate) were obtained from local commercial sources and analyzed for nutrient composition. The analyzed Ca and P concentrations were used to formulate the assay diets.

The recommended requirements of total Ca and aP for Ross 308 broiler finishers (25−35 d post-hatch) are 7.80 and 3.90 g/kg, respectively (Ross, 2019). Based on published values of digestible Ca and P in feed ingredients (Tables 1 and 2), equivalent SID Ca and SID P values were 4.25 and 3.91 g/kg, respectively. Most of the selected SID Ca (2.0−4.0 g/kg) and SID P (2.5−4.5 g/kg) concentrations of the diets used in this study were below the predicted target values based on previous findings for starters (David et al., 2021c) and growers (David et al., 2022) which suggested the true requirements were lower than the targets. The ingredient and calculated nutrient composition of broiler starter crumbles (4.4 g/kg SID Ca; 5.0 g/kg SID P) and grower pelleted diet (3.69 g/kg SID Ca; 3.5 g/kg SID P) offered to broilers from d 1 to 10 and d 11 to 24, respectively, are shown in Table 3. Fifteen experimental finisher diets based on corn-soybean meal were formulated in a 5 × 3 factorial arrangement with diets containing 5 concentrations of SID Ca and 3 concentrations of SID P (Table 4). Diets were formulated to contain 2.0, 2.5, 3.0, 3.5, and 4.0 g/kg SID Ca (corresponding to 3.7, 4.6, 5.5, 6.4, and 7.3 g/kg total Ca, respectively), and 2.5, 3.5, and 4.5 g/kg SID P (corresponding to 3.4, 4.9, and 6.4 g/kg total P, respectively) as indicated in Table 5. The concentration of total Ca ranged from 0.47 to 0.94 times the requirement for total Ca (Ross, 2019). All experimental diets were isoenergetic and isonitrogenous. Each diet

Table 1. Total and standardized ileal digestible (SID) calcium (Ca) content of feed ingredients.

Ingredients	Total Ca (g/kg)[1]	SID Ca digestibility (%)	SID Ca (g/kg)
Corn	0.08	50[2]	0.04
Soybean meal	3.40	54[3]	1.84
Dicalcium phosphate	260	36[4]	93.6
Limestone	410	55[4,5]	226

[1]Analyzed values.
[2]Assumed value.
[3]David et al. (2021a).
[4]David et al. (2019).
[5]Anwar et al. (2016c).

Table 2. Total and standardized ileal digestible (SID) phosphorous (P) contents of feed ingredients.

Ingredient	Total P (g/kg)[1]	SID P digestibility (%)	SID P (g/kg)
Corn	2.20	70[2]	1.54
Soybean meal	6.90	75[2]	5.18
Dicalcium phosphate	185	79[3]	146
Monosodium phosphate	270	67[4]	181

[1]Analyzed values.
[2]Mutucumarana et al. (2015a).
[3]van Harn et al. (2017).
[4]Shastak et al. (2012).

Table 3. Ingredient and calculated nutrient compositions of broiler starter (1−10 d post-hatch) and grower (11−24 d post-hatch) diets (g/kg, as fed basis).

Ingredient	Starter diet (1−10 d)	Grower diet (11−24 d)
Maize	560	615
Soybean meal	363	325
Dicalcium phosphate	10.1	5.97
Monosodium phosphate	6.70	-
Limestone	12.2	11.1
Sodium chloride	0.70	0.84
Sodium bicarbonate	1.00	4.08
DL Methionine	3.80	3.22
Lysine HCl	4.80	4.10
L Threonine	2.70	2.15
L Valine	1.30	0.94
Vitamin premix[1]	1.00	1.00
Trace mineral premix[2]	1.00	1.00
Choline chloride 60%	0.80	0.73
Soybean oil	31.1	24.9
Calculated composition		
Apparent metabolizable energy (kcal/kg)	3,000	3,050
Crude protein	220	205
Digestible protein	179	166
Starch	348	385
Crude fat	51.7	47.7
Crude fiber	28.1	27.8
Total Ca	9.00	7.27
SID Ca	4.43	3.69
Total P	6.80	4.70
Phytate P	1.92	2.03
Non-phytate P	4.88	2.67
SID P	5.00	3.50
Total Ca: Non-phytate P	1.85	2.72
SID Ca: SID P	0.89	1.05
Chloride	1.90	1.90
Sodium	2.30	1.90
Potassium	11.0	10.3
Choline (mg/kg)	1,700	1,600
Dig. threonine	8.60	7.70
Dig. alanine	8.10	7.78
Dig. valine	9.60	8.70
Dig. isoleucine	7.30	6.79
Dig. leucine	15.0	14.4
Dig. lysine	12.8	11.5
Dig. arginine	12.6	11.7
Dig. cysteine	2.92	2.83
Dig. methionine	6.58	5.87
Dig. met. + cysteine	9.50	8.70

[1]Supplied per kilogram of diet: vitamin A (trans-retinyl acetate), 12,000 IU; cholecalciferol, 4,000 IU; thiamine, 3 mg; riboflavin, 9 mg; pyridoxine, 10 mg; folic acid, 3 mg; biotin, 0.25 mg; cyanocobalamin, 0.02 mg; dl-α-tocopherol acetate, 80 IU; niacin, 60 mg; Ca-D pantothenate, 15 mg; menadione, 4 mg; choline chloride, 600 mg; Co, 0.25 mg; I, 1.5 mg; Mo, 0.25 mg; Se, 0.26 mg; Mn, 100 mg; Cu, 10 mg; Zn, 80 mg; Fe, 60 mg; antioxidant, 100 mg.
[2]Vitamin and mineral premix contained no calcium.
Abbreviations: Ca, calcium; Dig., digestible; P, phosphorous; SID, standardized ileal digestible.

Table 4. Ingredient composition of experimental diets (g/kg, as fed basis).

SID Ca	2.0			2.5			3.0			3.5			4.0		
SID P	2.5	3.5	4.5	2.5	3.5	4.5	2.5	3.5	4.5	2.5	3.5	4.5	2.5	3.5	4.5
SID Ca: SID P	0.80	0.57	0.45	1.00	0.71	0.56	1.20	0.86	0.66	1.40	1.00	0.78	1.60	1.14	0.89
Total Ca	3.69	3.69	3.69	4.60	4.60	4.60	5.51	5.51	5.52	6.42	6.42	6.42	7.33	7.33	7.34
Corn	676	673	661	671	668	657	667	664	652	662	659	648	658	655	643
Soybean meal	274	275	277	275	275	277	276	276	278	276	277	279	277	278	279
Dicalcium phosphate	0.28	0.28	0.28	0.30	0.30	0.30	0.32	0.32	0.32	0.34	0.34	0.34	0.37	0.37	0.37
Monosodium phosphate	0	5.54	11.1	0	5.54	11.1	0	5.54	11.1	0	5.54	11.1	0	5.54	11.1
Limestone	6.40	6.40	6.40	8.60	8.60	8.60	10.8	10.8	10.8	13.01	13.01	13.01	15.21	15.21	15.21
Sodium chloride	1.06	1.07	1.09	1.07	1.08	1.10	1.08	1.08	1.10	1.09	1.09	1.11	1.09	1.10	1.12
Sodium bicarbonate	4.01	0	0	4.00	0	0	4.00	0	0	3.99	0	0	3.99	0	0
DL Methionine	2.86	2.87	2.89	2.87	2.88	2.89	2.88	2.88	2.90	2.88	2.89	2.91	2.89	2.90	2.91
Lysine HCl	3.75	3.74	3.71	3.74	3.73	3.70	3.73	3.72	3.69	3.72	3.71	3.68	3.70	3.70	3.67
L Threonine	1.80	1.79	1.79	1.79	1.79	1.79	1.79	1.79	1.79	1.79	1.79	1.79	1.79	1.79	1.79
L Valine	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76
Vitamin premix - Ca-free[1]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix - Ca-free[1]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Choline chloride 60%	0.74	0.74	0.74	0.74	0.74	0.75	0.74	0.74	0.75	0.74	0.74	0.75	0.75	0.74	0.75
Titanium dioxide	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Soybean oil	21.6	22.7	26.6	23.2	24.2	28.1	24.7	25.8	29.6	26.2	27.3	31.2	27.8	28.9	32.7

[1]Supplied per kilogram of diet: vitamin A (trans-retinyl acetate), 12,000 IU; cholecalciferol, 4,000 IU; thiamine, 3 mg; riboflavin, 9 mg; pyridoxine, 10 mg; folic acid, 3 mg; biotin, 0.25 mg; cyanocobalamin, 0.02 mg; dl-α-tocopherol acetate, 80 mg; niacin, 60 mg; Ca-D pantothenate, 15 mg; menadione, 4 mg; choline chloride, 600 mg; Co, 0.25 mg; I, 1.5 mg; Mo, 0.25 mg; Se, 0.26 mg; Mn, 100 mg; Cu, 10 mg; Zn, 80 mg; Fe, 60 mg; antioxidant, 100 mg. Vitamin and mineral premix contained no calcium.
Abbreviations: Ca, calcium; P, phosphorous; SID Ca, standardized ileal digestible.

was separately mixed and pelleted. The diets were steam-conditioned at 70°C for 30 s and pelleted using a pellet mill (Model Orbit 15; Richard Sizer Ltd., Kingston-upon-Hull, UK) capable of manufacturing 180 kg of feed/h and equipped with a die ring with 3 mm holes and 35 mm thickness.

Birds

Day-old male broilers (Ross 308) were obtained from a commercial hatchery and raised on floor pens until d 24. The birds were fed the broiler starter crumbles from d 1 to 10 post-hatch and the broiler grower pellets from d 11 to

Table 5. Calculated and analyzed nutrient composition of experimental diets (g/kg, as fed basis).

SID Ca	2.0			2.5			3.0			3.5			4.0		
SID P	2.5	3.5	4.5	2.5	3.5	4.5	2.5	3.5	4.5	2.5	3.5	4.5	2.5	3.5	4.5
SID Ca: SID P	0.80	0.57	0.45	1.00	0.71	0.56	1.20	0.86	0.66	1.40	1.00	0.78	1.60	1.14	0.89
Total Ca	3.69	3.69	3.69	4.60	4.60	4.60	5.51	5.51	5.52	6.42	6.42	6.43	7.33	7.33	7.33
Non-phytate P	1.49	2.99	4.49	1.49	2.99	4.49	1.49	2.99	4.49	1.50	3.00	4.50	1.50	2.99	4.50
Total Ca: Non-phytate P	2.47	1.24	0.82	3.08	1.54	1.03	3.68	1.84	1.23	4.28	2.14	1.43	4.88	2.45	1.63
Dry matter	893	892	889	891	891	887	890	889	886	889	888	885	888	887	884
AME (kcal/kg)	3,100	3,100	3,100	3,100	3,100	3,100	3,100	3,100	3,100	3,100	3,100	3,100	3,100	3,100	3,100
Crude protein	185	185	185	185	185	185	185	185	185	185	185	185	185	185	185
Digestible protein	150	150	150	150	150	150	150	150	150	150	150	150	150	150	150
Starch	421	419	412	418	416	409	416	414	407	413	411	404	410	408	401
Crude fat	46.3	47.2	50.3	47.6	48.4	51.6	48.8	49.7	52.9	50.1	51.0	54.2	51.4	52.2	55.4
Crude fiber	26.9	26.8	26.7	26.8	26.8	26.6	26.7	26.7	26.5	26.7	26.6	26.5	26.6	26.6	26.4
SID Ca	2.00	2.00	2.00	2.50	2.50	2.50	3.00	3.00	3.00	3.50	3.50	3.50	4.00	4.00	4.00
Total P	3.43	4.92	6.41	3.43	4.92	6.41	3.43	4.92	6.41	3.43	4.92	6.41	3.43	4.92	6.41
Phytate P	1.94	1.94	1.93	1.94	1.93	1.92	1.93	1.93	1.92	1.93	1.92	1.91	1.92	1.92	1.91
SID P	2.50	3.50	4.50	2.50	3.50	4.50	2.50	3.50	4.50	2.50	3.50	4.50	2.50	3.50	4.50
Chloride	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sodium	1.97	1.98	3.07	1.97	1.98	3.07	1.97	1.98	3.07	1.97	1.98	3.08	1.97	1.98	3.08
Potassium	9.22	9.22	9.23	9.22	9.22	9.23	9.22	9.23	9.23	9.23	9.23	9.23	9.23	9.23	9.24
Choline (mg/kg)	1,500	1,500	1,500	1,500	1,500	1,500	1,500	1,500	1,500	1,500	1,500	1,500	1,500	1,500	1,500
Dig. threonine	6.80	6.80	6.80	6.80	6.80	6.80	6.80	6.80	6.80	6.80	6.80	6.80	6.80	6.80	6.80
Dig. alanine	7.34	7.34	7.31	7.33	7.33	7.30	7.32	7.32	7.29	7.31	7.31	7.28	7.30	7.29	7.27
Dig. valine	7.80	7.80	7.80	7.80	7.80	7.80	7.80	7.80	7.80	7.80	7.80	7.80	7.80	7.80	7.80
Dig. isoleucine	6.07	6.07	6.08	6.07	6.07	6.08	6.07	6.08	6.08	6.08	6.08	6.08	6.08	6.08	6.09
Dig. leucine	13.4	13.4	13.4	13.4	13.4	13.4	13.4	13.4	13.3	13.4	13.4	13.3	13.4	13.4	13.3
Dig. lysine	10.2	10.2	10.2	10.2	10.2	10.2	10.2	10.2	10.2	10.2	10.2	10.2	10.2	10.2	10.2
Dig. arginine	10.4	10.4	10.4	10.4	10.4	10.4	10.4	10.4	10.4	10.4	10.4	10.4	10.4	10.4	10.4
Dig. cysteine	2.67	2.67	2.66	2.67	2.66	2.65	2.66	2.66	2.65	2.66	2.66	2.65	2.65	2.65	2.64
Dig. methionine	5.33	5.33	5.34	5.33	5.34	5.35	5.34	5.34	5.35	5.34	5.35	5.36	5.35	5.35	5.36
Dig. methionine + cysteine	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Analyzed values[1]															
Dry matter	886	892	894	889	893	892	890	889	892	891	894	899	896	895	895
Total Ca	4.20	3.69	3.75	5.55	4.96	5.26	6.02	5.69	6.58	6.28	7.21	7.33	8.19	8.88	8.43
Total P	3.95	5.25	6.76	4.16	5.26	6.75	3.79	5.29	6.87	3.92	5.47	6.89	3.89	5.47	7.02

Abbreviations: AME, apparent metabolizable energy; Ca, calcium; Dig., digestible; P, phosphorous; SID, standardized ileal digestible.
[1]Samples were analyzed in triplicate.

24 post-hatch. On d 25, the birds were individually weighed and, 720 birds were selected and randomly allocated (mean ± SD, 1.31 ± 0.02 kg) to 90 grower cages (8 birds per cage). Each of the 15 experimental diets was offered ad libitum to 6 replicate cages of broilers from d 25 to 35 post-hatch. The birds had free access to water. Temperature was maintained at 31°C on d 1 and gradually reduced to 22°C by d 21 post-hatch. A lighting schedule of 20 h light per day was provided. Central ceiling extraction fans and wall inlet ducts controlled ventilation.

Measurements

Growth Performance Body weight and feed intake were recorded on a cage basis. Mortality was recorded daily. Feed conversion ratio (**FCR**) was corrected for the body weight of any bird that died during the experiment.

Ileal Digestibility and Apparent Total Tract Retention of Ca and P On d 35, six birds per replicate cage were euthanized by an intravenous injection (0.5 mL per kg body weight) of sodium pentobarbitone (Provet NZ Pty. Ltd., Auckland, New Zealand) and contents of the lower half of ileum were collected by flushing the contents gently with distilled water into plastic containers and processed as described by Ravindran et al. (2005). On d 31, excreta collection trays were introduced, and total excreta samples were collected during last 4 d, pooled within a cage and processed as described by David et al. (2019). Feed intake and dry excreta output were recorded during these 4 d to measure the total tract retention of Ca and P.

Bone Mineralization On d 35, the right tibia was removed from 6 birds per replicate (from the birds euthanized for ileal digesta) and immediately frozen at −20°C. Tibiae were cleaned from all adherent tissues and were kept frozen in airtight plastic bags until the measurements. Tibiae were oven dried at 105°C for 24 h, de-fatted by refluxing petroleum ether in a Soxhlet apparatus for 16 h, oven-dried at 105°C overnight for dry defatted bone weight determination, and ashed in ceramic crucibles for 24 h at 600°C for fat-free ash weight determination. Tibia ash content was expressed as a percentage of dry bone weight. Tibia Ca and P concentrations were determined and expressed as g/kg dried defatted bone.

Carcass Retentions of Ca and P At the start of the trial (d 25), 10 additional chicks were randomly selected and killed by cervical dislocation. At the end of experiment (d 35), 2 birds per replicate were randomly selected, fasted overnight, weighed, and killed with minimum blood loss. At both ages, feathers were removed using a defeathering machine, the carcass weight was recorded and defeathered carcasses were stored at −20° C. In this study, the term 'carcass' refers to the whole body without feathers. The frozen carcasses were cut into small pieces and minced twice to obtain homogenous subsamples.

Chemical Analysis

Ingredients were analyzed for DM (method 930.15; AOAC, 2016), ash (method 942.05; AOAC, 2016), nitrogen (968.06; AOAC, 2016), fat (AOAC 2003.06), crude fiber (AOAC 2002.04), Ca and total P (method 968.08D; AOAC, 2016) and phytate P (Caldwell, 1992). The concentrations of ash, Ca, and total P of tibia were determined using method 968.08D of AOAC (2016). The diet, ileal digesta, and excreta samples were analyzed for DM, Ca, total P, and titanium dioxide (Short et al., 1996). Subsamples of the minced carcass were analyzed for DM, Ca, and P.

Calculations

The apparent ileal digestibility coefficients (**AIDC**) of Ca and P were calculated using titanium marker ratios in the diet and ileal digesta (Ravindran et al., 1999) as indicated below. Analyzed values were used in digestibility and retention calculations.

$$\text{AIDC of Ca or P} = 1 - [(\text{Ti}_\text{I}/\text{Ti}_\text{O}) \times (M_\text{O}/M_\text{I})]$$

Where Ti_I is the titanium concentration in the diet, Ti_O is the titanium concentration in the ileal digesta, M_O is the concentration of Ca or P in the ileal digesta, and M_I is the concentration of Ca or P in the diet. All concentrations were expressed as g/kg DM.

Standardized ileal digestibility coefficients (**SIDC**) of Ca and P were then calculated, based on previously determined values for endogenous Ca (108 mg/kg DM intake, Anwar, 2017) and P (25 mg/kg DM intake, Mutucumarana and Ravindran, 2020) values, as follows:

$$\text{SIDC} = \text{AIDC} + (\text{IEL}/M_\text{I})$$

where IEL represents the ileal endogenous losses (mg/kg DM intake) of Ca or P.

The apparent total tract retention coefficient (**ATTRC**) of Ca and P (% intake) was calculated using the following equation:

$$\text{ATTRC of Ca or P} = [(M_I \times \text{FI}) - (M_E \times \text{EO})/M_I \times \text{FI}]$$

where FI is the feed intake of birds (g, DM basis), M_E is the concentration of Ca or P in the excreta (g/kg DM) and EO is the excreta output (g, DM basis).

The intake of SID Ca or P and the retained Ca or P (g/bird) was calculated using the following equations:

$$\text{Intake of SID Ca or P} = (\text{FI} \times M_I \times \text{SIDC})$$

$$\text{Retained Ca or P} = (\text{FI} \times M_I \times \text{ATTRC})$$

where FI is the feed intake of birds (g/bird, DM basis).

The retained Ca or P (g/bird) in the carcass was calculated using the following equation:

$$\text{Retained Ca or P} = \left[(Mc \times \text{CW})_{\text{D35}} - (Mc \times \text{CW})_{D25}\right]$$

Table 6. Analyzed nutrient and mineral composition of calcium and phosphorous (P) supplements (g/kg, as received basis).[1]

Nutrient	Corn	Soybean meal	Limestone	Dicalcium phosphate	Monosodium phosphate
Dry matter	901	896	1,000	969	996
Ash	12	65	996	848	-
Crude protein	75	475	-	-	-
Fat	39	12	-	-	-
Neutral detergent fiber	88	86	-	-	-
Calcium	0.08	3.4	410	260	-
Total P	2.2	6.9	0.56	185	270
Phytate	6.30	24.03	-	-	-
Phytate P[2]	1.76	6.73	-	-	-
Non-phytate P[3]	0.44	0.17	-	-	-
Sodium	< 0.05	< 0.05	< 0.50	0.71	192

[1]Samples were analyzed in duplicate.
[2]Values were calculated based on the assumption that a phytate molecule contains 28% of phytate P.
[3]Calculated as the difference between total P and phytate P.

where M_c is the concentration of Ca or P in the carcass (g per kg DM), CW is the carcass weight (g/bird) and D35 and D25 denote 35-day-old and 25-day-old birds, respectively.

Statistical Analysis

Data were analyzed using the General Linear Model (**GLM**) procedure of SAS (2019), with cage serving as the experimental unit. Two sets of analyses were conducted as reported by David et al. (2021c). First, as a factorial arrangement of treatments examining the effects of dietary concentrations of SID Ca and SID P and their interaction. The effect was considered significant at $P \leq 0.05$. Second, if the interaction or main effect was significant, then the parameter estimate for the second-order response surface model (**RSM**) was determined using GLM procedure of SAS (2019). All calculations started with the full model, but if needed, the model was reduced by removing parameter estimates that were not significant ($P > 0.05$) and the estimate was recalculated using the reduced model as described by González-Vega et al. (2016b). Linear and quadratic effects of both SID Ca and SID P and the interaction between SID Ca and SID P were included in the full model as follows:

$$Y = a + b \times \text{SID Ca} + c \times \text{SID Ca}^2 + d \times \text{SID P} + e \times \text{SID P}^2 + f \times \text{SID Ca} \times \text{SID P}$$

where Y is the dependent variable, a is the intercept, b, c, d, e, and f are the coefficients, and SID Ca and SID P are the concentrations (g/kg) of dietary SID Ca and SID P.

The concentrations of SID Ca at the maximum or minimum response values were calculated using the following equation:

$$\text{SID Ca}_{\text{min/max}}(\text{g/kg}) = [(-f \times \text{SID P}) - b)]/(2 \times c)$$

where SID $\text{Ca}_{\text{min/max}}$ is the concentration of SID Ca at the maximum or minimum response and SID P is the concentration of SID P in the diet.

The maximum response values were, therefore, calculated using the respective model equations with the concentrations of SID Ca at the maximum response for each concentration of SID P. The relationship between measured parameters was analyzed by Pearson correlations (SAS, 2019).

RESULTS

The analyzed nutrient composition of Ca and P supplements and the main ingredients is summarized in Table 6.

Determined concentrations of SID Ca and SID P of the 15 assay diets, in comparison with formulated values, are summarized in Table 7. The calculated SID Ca concentrations of most dietary treatments, in general, were representative of the determined SID Ca concentrations. However, the calculated and determined SID Ca concentrations of 4 diets (out of 15 diets) differed by 19 to 22%. The diets formulated to have 3.5 g/kg SID P with 2.5 or 3.0 or 3.5 g/kg SID Ca and 2.5 g/kg SID P with 3.5 g/kg SID Ca are these 4 diets. On the other hand, the calculated SID P concentrations of the experimental diets were generally close to determined SID P concentrations, with the differences ranging between 0 and 16%.

Growth Performance

Table 8 and Figure 1 present the weight gain, feed intake, and FCR of broilers fed the diets containing different SID Ca and SID P from d 25 to 35 d. There were interactions ($P < 0.001$) between SID Ca and SID P for all performance parameters. At the lowest SID Ca (2.0 g/kg) concentration, increasing concentrations of SID P reduced the weight gain and feed intake. In contrast, increasing SID P concentrations increased the weight gain and feed intake at the highest SID Ca concentration (4.0 g/kg), but had no effect on the weight gain at 2.5 and 3.0 g/kg SID Ca concentrations. At the 3.5 g/kg SID Ca concentration, the weight gain and feed intake were higher ($P < 0.05$) at 3.5 g/kg SID P. At 2.0 and 3.5 g/kg SID Ca concentrations, the FCR was unaffected by increasing concentrations of SID P. However, at the highest SID Ca concentration (4.0 g/kg), the FCR was improved by increasing concentrations of SID P. Predictions for SID Ca at maximum response were not calculated for any of the performance parameters due to the linear Ca response in the RSM.

Table 7. Comparison of calculated and determined[1] values of standardized ileal digestible (SID) calcium (Ca) and SID (P) of experimental diets (g/kg, as fed basis).

SID Ca	2.0			2.5			3.0			3.5			4.0		
SID P	2.5	3.5	4.5	2.5	3.5	4.5	2.5	3.5	4.5	2.5	3.5	4.5	2.5	3.5	4.5
SID Ca: SID P	0.80	0.57	0.45	1.00	0.71	0.56	1.20	0.86	0.66	1.40	1.00	0.78	1.60	1.14	0.89
Determined SID Ca	1.79	1.97	1.93	2.23	2.01	2.69	2.99	2.34	2.99	2.80	2.77	2.89	3.80	3.64	3.40
Determined SID P	2.78	3.95	5.22	2.59	3.67	4.97	2.42	3.42	4.74	2.40	3.25	4.67	2.38	3.37	4.50
Determined SID Ca: SID P	0.64	0.50	0.37	0.86	0.55	0.54	1.24	0.68	0.63	1.17	0.85	0.62	1.60	1.08	0.76
Difference (calculated minus determined)															
SID Ca	0.21	0.03	0.07	0.27	0.49	-0.19	0.01	0.66	0.01	0.70	0.73	0.61	0.20	0.36	0.60
SID P	−0.28	−0.45	−0.72	−0.09	−0.17	−0.47	0.08	0.08	−0.24	0.10	0.25	−0.17	0.12	0.13	0.00

[1]Dietary Ca or P concentration × Determined SID Ca or SID P for the respective experimental diet.

Standardized Ileal Ca and P Digestibility Coefficients, Intake of Both SID Ca and SID P, and the Ratio Between SID Ca and SID P Intakes

Data on SIDC of Ca and P, the intake of SID Ca and SID P and the ratio of SID Ca intake to SID P intake in 25 to 35-day-old birds are presented in Table 9 and Figure 2. The SIDC of Ca was influenced by the dietary SID P at 2.5 and 3.0 g/kg SID Ca, but not at other SID Ca concentrations resulting in a SID Ca × SID P interaction ($P < 0.001$). A similar trend was observed for the digestible Ca intake. No predictions were made for these parameters because of the linear Ca effect in the RSM.

The main effects of SID Ca and SID P were significant ($P < 0.001$) for SIDC of P and SID P intake, where both parameters increased with increasing dietary SID P concentrations. Both SIDC of P and SID P intake were higher ($P < 0.001$) at low SID Ca concentrations (2.0 and 2.5 g/kg). The reduced model was used to predict the minimum values for both SIDC of P and SID P intake. The predicted minimum SIDC of P at SID P concentrations of 2.5, 3.5, and 4.5 g/kg were 0.67, 0.70, and 0.74, at the SID Ca concentration of 3.88 g/kg. These

Table 8. Growth performance of broiler chickens fed diets containing different concentrations of standardized ileal digestible (SID) calcium (Ca) and SID phosphorous (P) from d 25 to 35[1].

SID Ca	SID P	Body weight gain (g/bird)	Feed intake (g/bird)	Feed conversion ratio
2.0	2.5	1,134^{bcde}	1,706^{abcd}	1.505^{bcd}
	3.5	1,122^{cdef}	1,693^{abcde}	1.504^{bcd}
	4.5	1,064^{f}	1,599^{f}	1.504^{bcd}
2.5	2.5	1,102^{def}	1,696^{abcde}	1.539^{ab}
	3.5	1,161^{abcd}	1,735^{ab}	1.495^{cde}
	4.5	1,114^{def}	1,671^{bcdef}	1.502^{bcd}
3.0	2.5	1,113^{def}	1,696^{abcde}	1.526^{abc}
	3.5	1,144^{bcde}	1,766^{a}	1.547^{a}
	4.5	1,148^{abcd}	1,642^{def}	1.463^{ef}
3.5	2.5	1,117^{def}	1,645^{cdef}	1.474^{def}
	3.5	1,212^{a}	1,740^{ab}	1.436^{f}
	4.5	1,143^{bcde}	1,652^{cdef}	1.446^{f}
4.0	2.5	1,079^{ef}	1,626^{ef}	1.521^{abc}
	3.5	1,187^{abc}	1,720^{abc}	1.453^{f}
	4.5	1,198^{ab}	1,730^{ab}	1.444^{f}
SEM[2]		23.7	26.9	0.0136
Main effects				
SID Ca				
2.0		1,107	1,666	1.505
2.5		1,126	1,701	1.512
3.0		1,135	1,701	1.512
3.5		1,157	1,679	1.452
4.0		1,155	1,692	1.473
SEM[2]		13.7	15.5	0.0078
SID P				
2.5		1,109	1,674	1.513
3.5		1,165	1,731	1.487
4.5		1,134	1,659	1.472
SEM[2]		10.6	12.0	0.0061
Probability, $P \leq$				
SID Ca		0.059	0.443	0.001
SID P		0.002	0.001	0.001
SID Ca × SID P		0.020	0.019	0.002

[1]Each value represents the mean of 6 replicates (8 birds per replicate).
[a-f]Means having different superscripts within the column are significantly different ($P < 0.05$).
[2]Pooled standard error of mean.

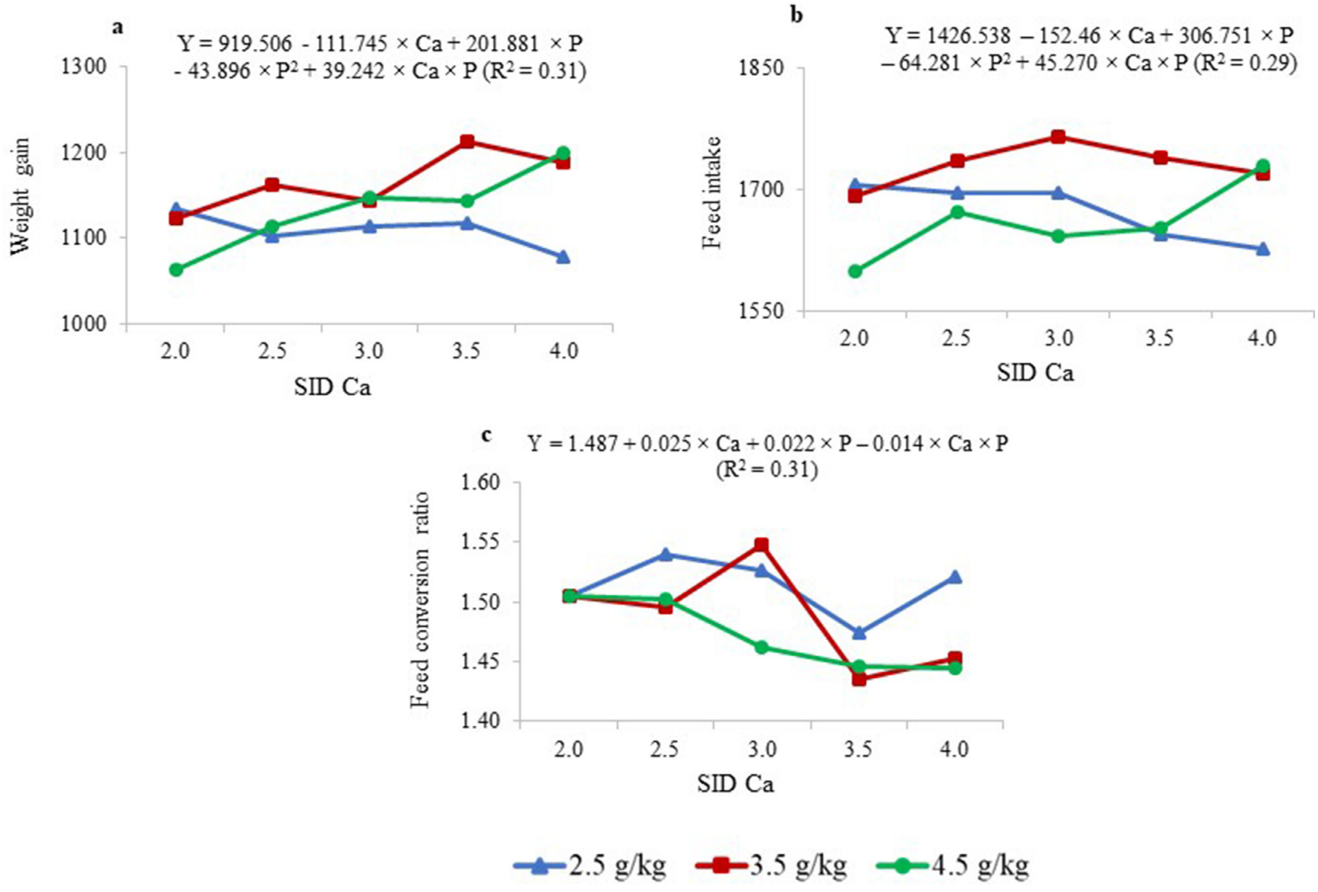

Figure 1. (a) Body weight gain (g/bird), (b) feed intake (g/bird), and (c) feed conversion ratio of broiler chickens fed different standardized ileal digestible (SID) calcium (Ca) and SID phosphorous (P) concentrations (2.5, 3.5 and 4.5 g/kg) from d 25 to 35.

values corresponded to SID Ca to SID P ratios of 1.55, 1.11, and 0.86, respectively. The predicted minimum SID P intake at SID P concentrations of 2.5, 3.5, and 4.5 g/kg were 3.90, 5.79, and 7.67, at the SID Ca concentration of 3.98 g/kg. These values corresponded to SID Ca to SID P ratios of 1.59, 1.14, and 0.88, respectively. The ratio of SID Ca intake to SID P intake increased ($P < 0.001$) by different magnitudes with increasing Ca concentrations and decreasing SID P concentrations, resulting in a SID Ca × SID P interaction ($P < 0.001$). The maximum values were not calculated for the ratio of SID Ca intake to SID P intake due to the linear Ca effect in the RSM.

Bone Mineralization

Table 10 and Figure 3 present the concentrations of ash, Ca, and P of tibia in 25 to 35 d old birds fed the diets containing different SID Ca and SID P. Based on the factorial arrangement of treatments, there was no interaction ($P > 0.05$) between SID Ca and SID P for any of the tibia parameters. Tibia ash was increased ($P < 0.05$) by increasing concentrations of SID P. Tibia Ca was not influenced ($P > 0.05$) by the main effects of either SID Ca or SID P and their interaction. Tibia P was reduced ($P < 0.05$) at the lowest SID P (2.5 g/kg). The predictions for maximum response were not made for all tibia parameters due to the linear Ca effect. The RSM for tibia Ca was not significant ($P = 0.415$; $R^2 = 0.06$) and, therefore, tibia Ca was not shown as a figure.

Coefficients of Apparent Total Tract Retention and Retained Ca and P

Table 11 and Figure 4 present the total tract retained Ca and P in 35-day-old birds fed diets containing different SID Ca and SID P. The ATTRC of Ca decreased ($P < 0.05$) with increasing SID Ca concentrations. Total tract retained Ca (g/bird) was influenced by increasing concentrations of SID P at 3.0 to 4.0 g/kg SID Ca concentrations, resulting in a SID Ca × SID P interaction ($P < 0.05$). At 3.0 g/kg SID Ca, the retained Ca was the lowest at 4.5 g/kg SID P, whereas at 3.5 and 4.0 g/kg SID Ca, the retained Ca was the lowest at 2.5 g/kg SID P. The ATTRC of P decreased ($P < 0.01$) with increasing SID P concentrations. The retained P (g/bird) increased with increasing SID P concentration, but the magnitude of increment was greater at 4.0 g/kg SID Ca, resulting in a SID Ca × SID P interaction ($P < 0.01$). The ratio between retained Ca and retained P was increased with decreasing SID P concentration, but the magnitude of increment was greater as the SID Ca increased, resulting in a SID Ca × SID P interaction ($P < 0.001$). The predictions for maximum responses could

Table 9. Standardized ileal digestibility coefficient (SIDC) of calcium (Ca) and phosphorous (P), intake (g/bird) of standardized ileal digestible (SID) Ca and SID P, and the ratio of SID Ca intake to SID P intake, in broiler chickens fed different concentrations (g/kg) of SID Ca and SID P from d 25 to 35[1]

SID Ca	SID P	SIDC of Ca	SID Ca intake	SIDC of P	SID P intake	SID Ca intake: SID P intake
2.0	2.5	0.52^{abc}	3.05^{f}	0.80	4.75	0.64^{ef}
	3.5	0.57^{a}	3.33^{ef}	0.83	6.69	0.50^{g}
	4.5	0.56^{ab}	3.09^{f}	0.85	8.35	0.37^{h}
2.5	2.5	0.48^{cdef}	3.78^{de}	0.74	4.39	0.86^{c}
	3.5	0.43^{f}	3.49^{def}	0.77	6.37	0.55^{fg}
	4.5	0.57^{a}	4.50^{bc}	0.81	8.30	0.54^{fg}
3.0	2.5	0.51^{abcd}	4.72^{bc}	0.69	4.11	1.15^{b}
	3.5	0.43^{f}	4.10^{cd}	0.72	6.03	0.68^{de}
	4.5	0.55^{ab}	4.91^{b}	0.77	7.79	0.63^{ef}
3.5	2.5	0.45^{def}	4.61^{bc}	0.68	3.95	1.17^{b}
	3.5	0.45^{ef}	4.82^{b}	0.68	5.65	0.85^{c}
	4.5	0.46^{cdef}	4.78^{b}	0.76	7.71	0.62^{ef}
4.0	2.5	0.50^{bcde}	6.16^{a}	0.68	3.87	1.60^{a}
	3.5	0.48^{cdef}	6.27^{a}	0.70	5.81	1.08^{b}
	4.5	0.45^{ef}	5.88^{a}	0.73	7.79	0.75^{cd}
SEM[2]		0.023	0.224	0.016	0.174	0.037
Main effects						
SID Ca						
2.0		0.55	3.16	0.82^{a}	6.60^{a}	0.50
2.5		0.49	3.92	0.77^{b}	6.35^{a}	0.65
3.0		0.50	4.58	0.73^{c}	5.98^{b}	0.82
3.5		0.45	4.74	0.71^{c}	5.77^{b}	0.88
4.0		0.47	6.10	0.70^{c}	5.82^{b}	1.15
SEM		0.013	0.129	0.009	0.100	0.022
SID P						
2.5		0.49	4.46	0.72^{c}	4.21^{c}	1.09
3.5		0.47	4.40	0.74^{b}	6.11^{b}	0.73
4.5		0.52	4.63	0.79^{a}	7.99^{a}	0.58
SEM		0.010	0.100	0.007	0.078	0.017
Probabilities, $P \leq$						
SID Ca		0.001	0.001	0.001	0.001	0.001
SID P		0.006	0.253	0.001	0.001	0.001
SID Ca × SID P		0.001	0.032	0.833	0.957	0.001

[1]Each value represents the mean of 6 replicates (8 birds per replicate).
[a-h]Means having different superscripts within the column are significantly different ($P < 0.05$).
[2]Pooled standard error of mean.

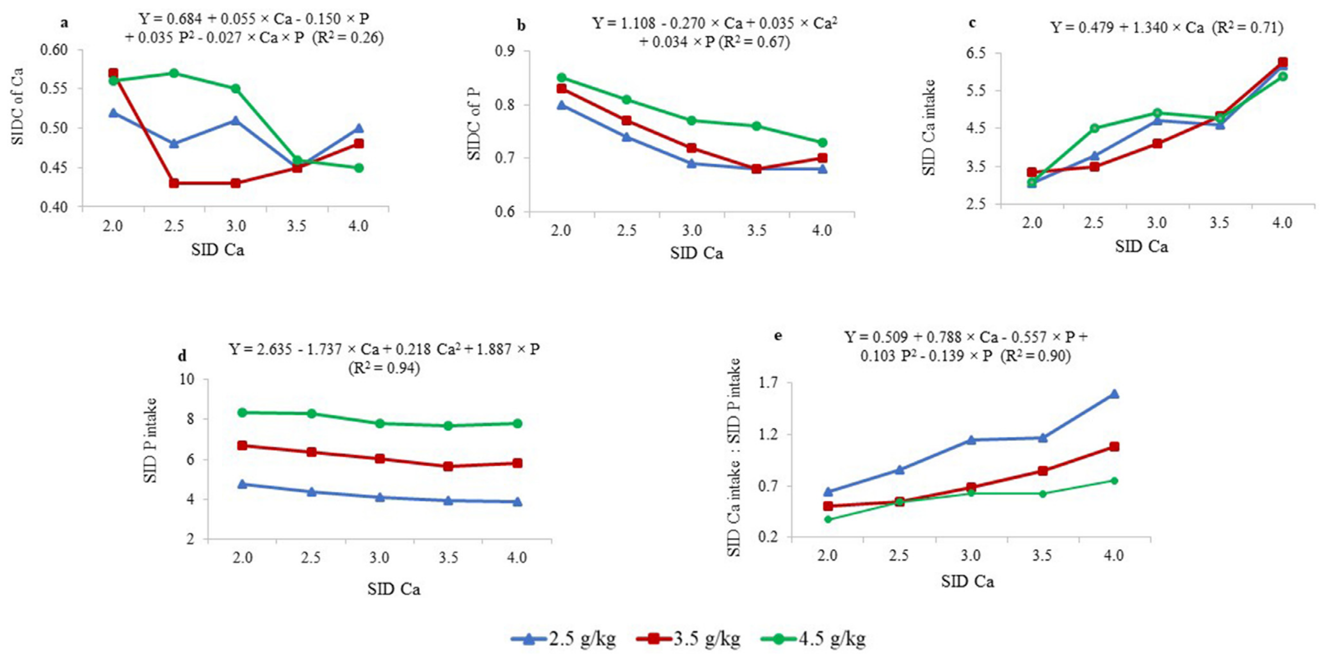

Figure 2. (a) Standardized ileal digestibility coefficients (SIDC) of calcium (Ca) and (b) phosphorous (P); intake (g/bird) of (c) standardized ileal digestible (SID) Ca and (d) SID P; and (e) ratio of SID Ca intake: SID P intake, of broiler chickens fed different concentrations of SID Ca and SID P (2.5, 3.5, and 4.5 g/kg) from d 25 to 35.

Table 10. Concentration of ash, calcium (Ca) and phosphorous (P) in tibia (g/kg dried defatted matter) in broiler chickens fed diets containing different concentrations (g/kg) of standardized ileal digestible (SID) Ca and SID P from d 25 to 35[1]

SID Ca	SID P	Tibia ash	Tibia Ca	Tibia P
2.0	2.5	393	136	64.9
	3.5	395	136	66.1
	4.5	404	137	67.7
2.5	2.5	391	135	64.1
	3.5	399	141	64.8
	4.5	409	141	68.3
3.0	2.5	395	133	63.0
	3.5	411	140	67.5
	4.5	417	144	68.3
3.5	2.5	389	134	61.8
	3.5	412	142	66.7
	4.5	409	130	62.9
4.0	2.5	384	135	61.9
	3.5	406	139	66.3
	4.5	425	146	69.1
SEM[2]		5.7	4.2	1.77
Main effects				
SID Ca				
2.0		397	136	66.2
2.5		400	139	65.8
3.0		408	139	66.2
3.5		403	135	63.8
4.0		405	140	65.8
SEM[2]		3.3	2.4	1.02
SID P				
2.5		391[c]	135	63.1[b]
3.5		404[b]	139	66.3[a]
4.5		413[a]	139	67.3[a]
SEM[2]		2.5	1.9	0.79
Probability, $P \leq$				
SID Ca		0.184	0.633	0.435
SID P		0.001	0.107	0.001
SID Ca × SID P		0.211	0.440	0.485

[1]Each value represents the mean of 6 replicates (6 birds per replicate).
[a-c]Means having different superscripts within the column are significantly different ($P < 0.05$).
[2]Pooled standard error of means.

not be made for total tract retention parameters due to the linear Ca effect in the RSM.

Carcass Retention of Ca and P

Data on the retention of Ca and P in the carcass of 35 d old broilers fed diets containing different SID Ca and SID P concentrations are presented in Table 12 and Figure 5. A reduced model was used for these parameters. Increasing concentrations of SID P influenced the retention of carcass Ca and P at the SID Ca between 3.0 and 4.0 g/kg, resulting in a SID Ca × SID P interaction ($P < 0.01$). Retention of both carcass Ca and carcass P were increased with increasing SID P concentration at 3.0 and 4.0 g/kg SID Ca and was highest at 4.0 g/kg SID Ca when the SID P was 3.5 or 4.5 g/kg.

Summary of SID Ca and SID P Requirement Estimates for Broiler Starters, Growers, and Finishers

A summary of the estimated requirements of SID Ca, SID P, and their ratio for growth performance and tibia ash in broiler starters (David et al., 2021c), growers (David et al., 2022), and finishers (current study) is presented in Table 13. It could be seen that the estimated requirements of SID P (5.0, 3.5, and 3.5 g/kg for starters, growers, and finishers, respectively) to maximize weight gain and tibia ash was similar within each growth phase. The SID P requirement for growth performance and tibia ash was similar between the growers and finishers and 30% lower than starters. The SID Ca requirements for maximum weight gain of broiler starters, growers, and finishers were 3.32, 3.05, and 3.50 g/kg, respectively (corresponding to 7.0, 6.1, and 6.4 g/kg total Ca), and for maximum tibia ash was 4.51, 3.69, and 3.0 to 3.5 g/kg, respectively (corresponding to 9.2,

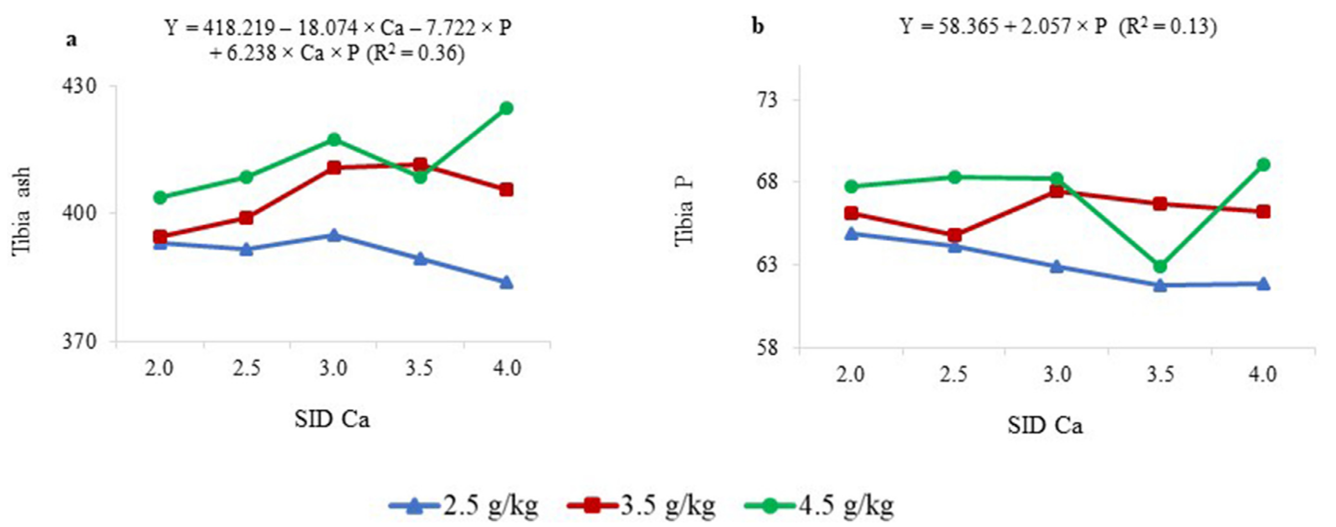

Figure 3. Concentrations (g/kg dried defatted matter) of (a) ash and (b) phosphorous (P) of tibia in broiler chickens fed different concentrations (g/kg) of standardized ileal digestible (SID) Ca and SID P (2.5, 3.5, and 4.5 g/kg) from d 25 to 35.

Table 11. Apparent total tract retention coefficients (ATTRC) of calcium (Ca) and phosphorous (P) and retained (g/bird) Ca and P in 35-dayold broilers fed diets containing different concentrations of (g/kg) standardized ileal digestible (SID) Ca and SID phosphorous (P)[1]

SID Ca	SID P	ATTRC of Ca	Retained Ca	ATTRC of P	Retained P	Retained Ca: retained P ratio
2.0	2.5	0.78	4.59[i]	0.85	5.09[e]	0.90[g]
	3.5	0.78	4.59[i]	0.79	6.42[d]	0.72[i]
	4.5	0.76	4.24[i]	0.76	7.42[c]	0.57[j]
2.5	2.5	0.77	6.13[h]	0.85	5.04[e]	1.22[d]
	3.5	0.78	6.33[gh]	0.80	6.62[d]	0.96[f]
	4.5	0.77	6.02[h]	0.75	7.65[bc]	0.79[h]
3.0	2.5	0.76	7.01[ef]	0.86	5.11[e]	1.37[c]
	3.5	0.76	7.28[de]	0.81	6.77[d]	1.08[e]
	4.5	0.75	6.72[fg]	0.76	7.63[bc]	0.88[g]
3.5	2.5	0.72	7.35[de]	0.85	4.93[e]	1.49[b]
	3.5	0.73	7.92[c]	0.80	6.69[d]	1.18[d]
	4.5	0.73	7.50[d]	0.77	7.80[b]	0.96[f]
4.0	2.5	0.73	9.04[b]	0.84	4.80[e]	1.88[a]
	3.5	0.74	9.68[a]	0.81	6.65[d]	1.46[b]
	4.5	0.74	9.72[a]	0.78	8.26[a]	1.18[d]
SEM[2]		0.011	0.143	0.007	0.132	0.017
Main effects						
SID Ca						
2.0		0.78[a]	4.47	0.80	6.31	0.73
2.5		0.77[ab]	6.16	0.80	6.43	0.99
3.0		0.76[b]	7.00	0.81	6.50	1.11
3.5		0.73[c]	7.59	0.81	6.47	1.21
4.0		0.74[c]	9.48	0.81	6.57	1.51
SEM[2]		0.006	0.082	0.004	0.076	0.010
SID P						
2.5		0.75	6.82	0.85[a]	4.99	1.37
3.5		0.76	7.16	0.80[b]	6.63	1.08
4.5		0.75	6.84	0.76[c]	7.75	0.88
SEM[2]		0.005	0.064	0.003	0.059	0.007
Probability, $P \leq$						
SID Ca		0.001	0.001	0.278	0.174	0.001
SID P		0.445	0.001	0.001	0.001	0.001
SID Ca × SID P		0.967	0.023	0.093	0.006	0.001

[1]Each value represents the mean of 6 replicates (6 birds per replicate).
[a-j]Means having different superscripts within the column are significantly different ($P < 0.05$).
[2]Pooled standard error of mean.

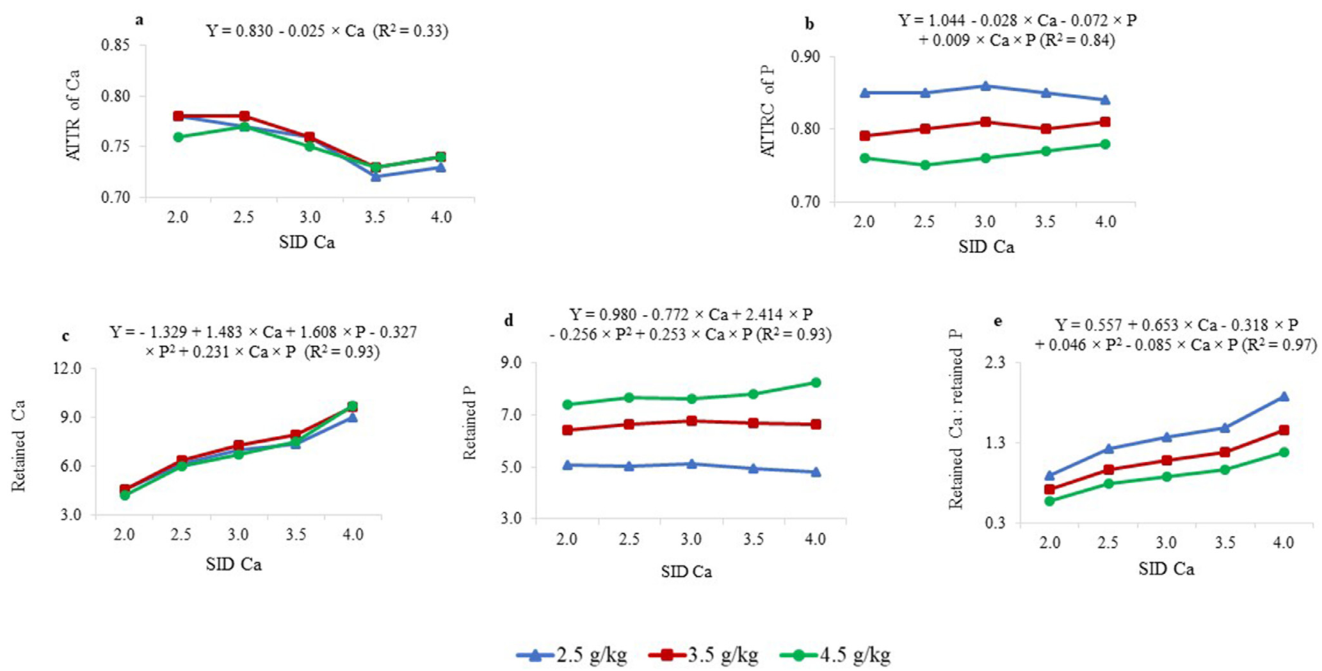

Figure 4. Apparent total tract retention coefficient (ATTRC) of (a) Ca and (b) phosphorous (P); retained (g/bird) (c) Ca and (d) P; (e) ratio of retained Ca to retained P, of broiler chickens fed different concentrations (g/kg) of standardized ileal digestible (SID) Ca and SID P (2.5, 3.5, and 4.5 g/kg) from d 25 to 35.

Table 12. Concentration (g/kg) and retention (g/bird) of calcium (Ca) and phosphorous (P) in the carcass of 35-day-old broilers fed diets containing different concentrations of (g/kg) standardized ileal digestible (SID) Ca and SID P.[1]

SID Ca	SID P	Carcass composition[2]		Carcass retention[3]	
		Ca	P	Ca	P
2.0	2.5	12.9[fg]	11.3[def]	4.36[de]	4.08[ef]
	3.5	13.3[efg]	11.7[bcde]	4.69[cde]	4.43[bcde]
	4.5	14.1[bcdef]	12.1[abcd]	5.15[bcde]	4.57[bcde]
2.5	2.5	13.5[defg]	11.3[def]	5.38[bcd]	4.61[bcde]
	3.5	14.1[bcdef]	11.6[cde]	5.22[bcd]	4.33[cdef]
	4.5	13.9[cdefg]	11.6[cde]	5.48[bcd]	4.68[bcde]
3.0	2.5	12.8[fg]	10.8[f]	4.77[cde]	4.14[def]
	3.5	14.4[bcdef]	11.6[cde]	5.84[bc]	4.68[bcde]
	4.5	15.5[ab]	12.4[ab]	6.06[b]	4.80[bc]
3.5	2.5	15.3[abc]	12.2[abc]	6.04[b]	4.73[bcd]
	3.5	13.3[efg]	11.2[ef]	4.91[cde]	4.26[cdef]
	4.5	15.0[abcd]	12.1[abc]	6.24[ab]	4.98[ab]
4.0	2.5	12.2[g]	10.6[f]	4.11[e]	3.75[f]
	3.5	14.7[abcde]	12.0[bcd]	6.11[b]	5.00[ab]
	4.5	16.3[a]	12.8[a]	7.29[a]	5.60[a]
SEM[4]		0.58	0.28	0.425	0.225
Main effects					
SID Ca					
2.0		13.5	11.7	4.73	4.36
2.5		13.8	11.5	5.36	4.54
3.0		14.2	11.6	5.56	4.54
3.5		14.5	11.8	5.73	4.65
4.0		14.4	11.8	5.84	4.78
SEM[4]		0.33	0.16	0.245	0.130
SID P					
2.5		13.3	11.2	4.93	4.26
3.5		13.9	11.6	5.35	4.54
4.5		15.0	12.2	6.04	4.92
SEM[4]		0.26	0.12	0.190	0.101
Probability, $P \leq$					
SID Ca		0.137	0.592	0.018	0.225
SID P		0.001	0.001	0.001	0.001
SID Ca × SID P		0.003	0.001	0.005	0.002

[1]Each value represents the mean of 6 replicates (2 birds per replicate). The term 'carcass' refers to the whole body without feathers.

[a-g]Means having different superscripts within the column are significantly different ($P < 0.05$).

[2]Concentration of Ca and P in the carcass of 25 d old bird is 13.7 and 11.2, respectively.

[3]Ca and P retained in the carcass of 25 d old bird is 4.66 and 3.81 g/bird, respectively, and these values are deducted from the total retained Ca or P at d 35.

[4]Pooled standard error of mean.

7.3, and 5.5−6.4 g/kg total Ca approximately), at recommended SID P concentrations.

DISCUSSION

In general, the analyzed concentration of proximate components, phytate, Ca and P of corn, soybean meal, limestone, dicalcium phosphate, and monosodium phosphate were within the range reported in the literature (NRC, 1994, 2012; Browning and Cowieson, 2014; Mutucumarana et al., 2014b,c; 2015b). It must be pointed out that the same batches of limestone and dicalcium phosphate used in the broiler starter (David et al., 2021c) and grower (David et al., 2022) requirement studies were utilized in the current experiment. In addition, the same batches of corn and soybean meal used in the broiler grower study were used in the current study.

As also observed in the broiler starter (David et al., 2021c) and grower (David et al., 2022) studies, the calculated SID Ca and SID P concentrations of most dietary treatments, in general, were comparable to the determined SID concentrations, providing confidence in the published values for the SID Ca and SID P of ingredients.

The calculated SID Ca values of most dietary treatments were, in general, slightly higher than the determined SID Ca values in the current study. However, the determined SID P values were slightly above the calculated SID P when diets were formulated at and below 2.5 g/kg dietary SID Ca while the determined SID P values were slightly lower than the calculated values when diets were formulated at and above 3.0 g/kg dietary SID Ca, except at 4.5 g/kg SID P, perhaps suggesting reduced P digestibility at higher Ca concentrations. Excess Ca (≥ 3.0 g/kg) might have resulted a formation of Ca-P complex (Selle et al., 2009) in the digestive tract, which would have resulted in lower determined SID P values. A similar trend was observed in the previous study with broiler growers above the threshold of 2.90 g/kg dietary SID Ca (David et al. 2022).

Requirement of SID P

In the current study, three SID P concentrations were used to determine the SID Ca and SID P requirements in 25 to 35-day-old broilers. As stated earlier, the test range (2.5−4.5 g/kg) was below and above the Ross (2019) recommendation for dietary P concentration (3.90 g/kg aP). Based on the present data, a SID P concentration of 3.5 g/kg is recommended for broiler finishers (25−35 d post-hatch) at 3.5 to 4.0 g/kg SID Ca concentrations. This recommendation is similar to that predicted for broiler growers (3.5 g/kg SID P; David et al., 2022), but lower than that for broiler starters (5.0 g/kg SID P; David et al., 2021c).

Requirement for SID Ca to Maximize Growth Performance

The SID Ca concentration at maximum response for growth performance parameters was not predicted due to the linear Ca response in the RSM. The present findings suggest that the effects of SID Ca on growth performance vary depending on dietary SID P concentrations. Accordingly, SID Ca concentrations of 2.0, 3.5, and 3.0 to 4.0 g/kg may be recommended for broiler finishers when the dietary SID P is 2.5, 3.5, and 4.5 g/kg, respectively. However, the factorial analysis of treatments indicate that the maximum weight gain was observed at 3.5 g/kg SID Ca and 3.5 g/kg SID P, and this combination may be recommended for the maximum growth performance of broiler finishers. Recommendations of SID Ca and SID P have been made for the maximum

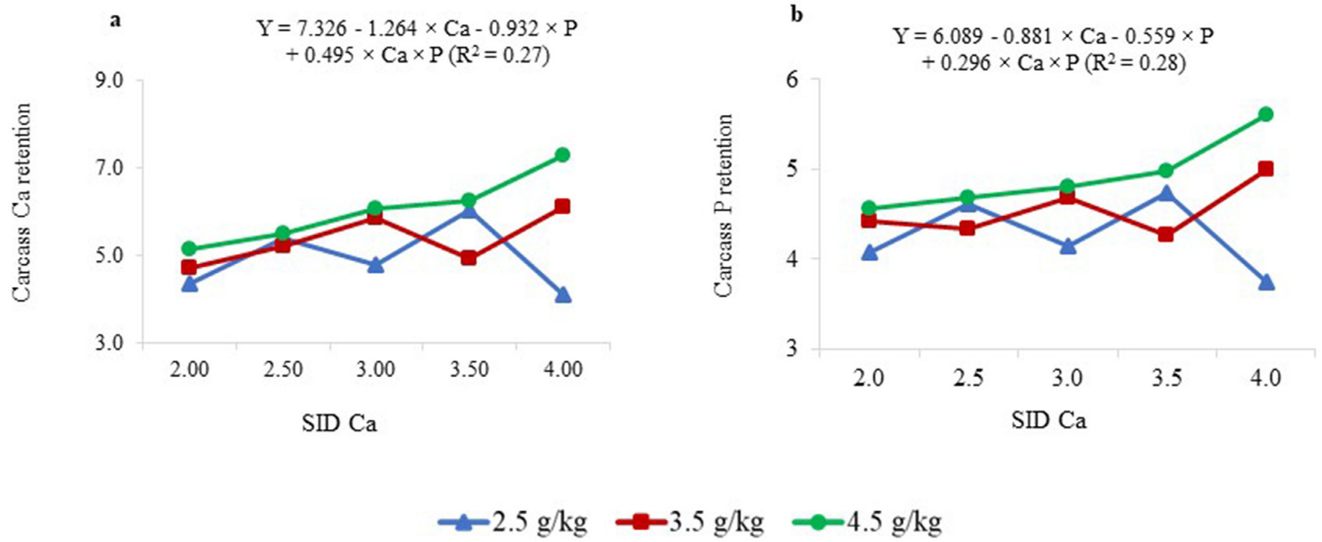

Figure 5. Carcass retention (g/bird) of (a) calcium (Ca), and (b) phosphorous (P) in 35-day-old broiler chickens fed different concentrations (g/kg) of standardized ileal digestible (SID) Ca and SID P (2.5, 3.5, and 4.5 g/kg).

performance in broiler starters and growers in previous studies where the requirement of SID Ca was 3.32 (David et al., 2021c) and 3.05 (David et al., 2022) g/kg, respectively, and the requirement of SID P was 5.0 and 3.5 g/kg, respectively. Overall, the growth of broiler finishers was maximized at 3.5 g/kg SID Ca (equivalent to 6.4 g/kg total Ca) which is below the current Ross (2019) Ca recommendation (7.8 g/kg total Ca).

Requirements for SID Ca to Maximize SIDC and Intake of Ca and P

The SIDC of Ca was influenced by dietary SID P concentrations only at 2.5 and 3.0 g/kg SID Ca concentrations where the highest digestibility was recorded at the highest SID P (4.5 g/kg). At 2.5 g/kg SID Ca and 4.5 g/kg SID P, the digestibility value was similar to those at the lowest SID Ca (2.0 g/kg), highlighting a need to increase dietary SID P concentration when the SID Ca is increased. However, at 4.5 g/kg SID P, the SIDC of Ca was reduced at higher SID Ca (3.5 and 4.0 g/kg) concentrations and this may be attributed to the formation of insoluble Ca-P complexes in the digestive tract due to excess Ca (Selle et al., 2009). A similar effect of excess Ca has been reported in our previous (David et al., 2022) and other (Walk et al., 2021; 2022a, b) studies. For the SIDC of Ca, although the linear SID P effect was not significant in the full model, it was included in the reduced model (Figure 2a) as the interaction between SID Ca and SID P was significant.

The digestibility and intake of SID P were influenced by the main effects of SID Ca and SID P. A positive correlation was observed between SIDC of P and SID P intake (r = 0.65; $P < 0.001$) where both parameters increased with increasing SID P concentrations. These findings are in agreement with the findings of our previous studies with broiler starters (David et al., 2021c) and growers (David et al., 2022). This finding suggests

Table 13. Summary of the standardized ileal digestible (SID) calcium (Ca) and phosphorous (P) requirements for broiler starter, grower, and finisher.

	Starter (1–10 d)[1]	Grower (11–24 d)[2]	Finisher (25–35 d)[3]
Weight gain			
SID Ca (g/kg)	3.32	3.05	3.50
SID P (g/kg)	5.00	3.50	3.50
SID Ca : SID P	0.66	0.87	1.00
Tibia ash			
SID Ca	4.51	3.69	3.00–3.50
SID P	5.00	3.50	3.50
SID Ca : SID P	0.90	1.05	0.86–1.00
Ross (2019) recommendations[4]			
SID Ca	4.40 (9.6)	4.03 (8.7)	4.25 (7.8)
SID P	5.40	4.83	3.91
SID Ca : SID P	0.81	0.83	1.09

[1]David et al. (2021c).
[2]David et al. (2022).
[3]Current work.
[4]Calculated equivalent SID values based on recommendations for total Ca (given in parenthesis, g/kg) and available P.

that the P digestibility increases with increasing dietary SID P (within a range of 2.5−6.0 g/kg), regardless of the age of broilers. Increasing SID Ca concentrations negatively affected the digestibility and intake of SID P and, as noted above, this effect may be explained by the formation of insoluble Ca-P complexes in the digestive tract. The negative influence of increasing dietary Ca on ileal P digestibility has been reported previously (Mutucumarana et al. 2014a; Walk et al., 2021; 2022a, b). Based on the RSM, the concentration of SID Ca that minimized SIDC of P and SID P intake were 3.88 and 3.98 g/kg, respectively, regardless of SID P concentrations in the current study, further confirming the negative effect of high SID Ca (above 3.5 g/kg) on the absorption of dietary P.

Requirements for Digestible Ca to Maximize Bone Mineralization

In the current study, tibia ash was positively correlated with tibia Ca (r = 0.53; $P < 0.001$) and tibia P (r = 0.70; $P < 0.001$). Tibia ash was increased with increasing SID P concentrations, a finding is similar to the trend observed with broiler growers (David et al., 2022). Similar to growers, the lowest concentration of SID P (2.5 g/kg) reduced the concentrations of tibia P. Curiously, dietary SID Ca concentration had no influence on tibia parameters, suggesting that the bone mineralization was unaffected within the 2.0 to 4.0 g/kg SID Ca range. Recommendations have been made for the requirement of SID Ca for maximum tibia ash in broiler starters and growers as 4.51 g/kg (David et al., 2021c) and 3.69 g/kg (David et al., 2022), respectively, using the RSM. However, in the current study, no predictions were made for tibia parameters of broiler finishers as the effect of Ca was linear in the RSM. It is worth noting that linear Ca effects were also observed for growth performance parameters in the current study. These observations imply that the highest level of Ca tested in the study may not be high enough and that the requirement of dietary SID Ca for maximization of these parameters, therefore, depends on the dietary SID P concentration when the dietary SID Ca is within 2.0 to 4.0 g/kg. Tibia ash was higher in birds fed 4.5 g/kg SID P and was unaffected by dietary SID Ca concentrations. Based on overall findings, a combination of 3.5 g/kg SID P and 3.0 to 3.5 g/kg SID Ca may be recommended for the optimum tibia ash. This estimate is comparable to the recent findings of Walk et al. (2022b) who proposed a SID Ca concentration of 3.7 g/kg for the maximum tibia ash in 25 to 42-day-old Arbor Acres plus broilers. The current results also show that the tibia ash would be increased if both SID Ca and SID P are increased. Recommended SID P for the maximum tibia ash in broiler starters and growers in the previous studies were 5.0 and 3.5 g/kg, respectively. These findings show that the SID P requirement to optimize the tibia ash in broiler finishers is 30% lower than that in starters while comparable to that in growers.

Requirements for SID Ca to Maximize Total Tract Retention of Ca and P

The negative effect of increasing dietary SID Ca concentrations on the ATTRC of Ca may be explained, at least in part, by the formation of insoluble Ca-phytate complex making Ca unavailable for absorption. The higher ATTRC of Ca (0.72−0.78) than the corresponding SIDC (0.43−0.57) is suggestive of possible absorption of Ca in the hindgut of broiler finishers. However, there is no evidence in published literature for post-ileal absorption of Ca in poultry. The findings on retained Ca (g/bird) further confirm that increasing dietary SID Ca requires an increased dietary SID P for higher Ca retention. According to the current finding, the SID P of 3.5 g/kg is required when the SID Ca concentration is between 3.0 and 4.0 g/kg.

At 2.5 and 3.5 g/kg SID P, the ATTRC of P (0.80−0.85) was higher than the SIDC of P (0.72−0.74) which again suggests post ileal absorption. However, the ATTRC (0.76) and SIDC (0.79) were similar at 4.5 g/kg SID P and, this retention value was lower than those at other SID P concentrations, suggesting that SID P lower than 4.5 g/kg must be considered for maximum P retention in broiler finishers. The ATTRC of P was negatively correlated (r = −0.40; $P < 0.001$) with the SIDC of P in the current study. Clearly the retained P increased with increasing SID P concentrations, regardless of dietary SID Ca. However, the retained P was the highest at 4.5 g/kg SID P when the dietary SID Ca was 4.0 g/kg, confirming higher requirement for dietary SID P when the SID Ca is high. Furthermore, the existence of a positive correlation (r = 0.42; $P < 0.001$) between retained P (g/bird) and tibia P shows that a significant portion of the retained P is deposited in the skeleton. The retained P (g/bird) was also positively correlated with weight gain (r = 0.40; $P < 0.001$), indicating that the retained P was utilized for the growth of broilers as P utilization is highly correlated with muscle protein synthesis (Xue et al., 2016).

For the ATTRC of P, although the linear SID Ca effect was not significant in the full RSM model, it was included in the reduced model as the interaction between SID Ca and SID P was significant. Similarly, for total tract retained Ca, although the linear SID P effect was not significant in the full model, it was included in the reduced model as the interaction between SID Ca and SID P was significant.

In the current study, the ratio of retained Ca and retained P was positively correlated (r = 0.94; $P < 0.001$) with the ratio of SID Ca intake to SID P intake, demonstrating the close association between Ca and P in their intake and retention in the body. At 3.5 g/kg dietary SID P, the ratio ranged from 0.72 to 1.46 depending on the dietary SID Ca concentration which is close to the range of ratios (0.72−1.23) reported for broiler growers (David et al., 2022) at 3.5 g/kg SID P.

Requirements for SID Ca to Maximize Carcass Retention of Ca and P

The Ca concentration of carcasses of 25-d and 35-day-old broilers was 13.7 and 10.1−17.7 g/kg, respectively. The use of different dietary Ca and P concentrations resulted in a wide range of carcass Ca concentrations in 35-day-old broilers. Carcass Ca concentrations of 16.9 and 14.7 g/kg in 22-d and 33-day-old birds, respectively, were reported by Caldas et al. (2019). Carcass P concentrations of 25-d and 35-day-old broilers birds in the current study were 11.2 and 9.6-13.5 g/kg, respectively. Similarly, Caldas et al. (2019) reported a carcass P concentration of 13.7 and 12.4 g/kg in 22-d and 35-day-old broilers.

Correlations between total tract Ca and P retentions (g/bird) and retained (g/bird) carcass Ca (r = 0.32; $P < 0.01$) and P (r = 0.44; $P < 0.001$) were positive, but weak. These poor correlations may be explained by the fact that the carcass and total tract retentions were measured at 2 different durations, with carcass retention being measured over 10 d (25−35 d post-hatch) and total tract retention over only 4 d (31−35 d post-hatch). However, Ca and P retention in the carcass had no correlation with Ca and P concentrations of tibia. Carcass Ca retention was highly positively correlated (r = 0.92; $P < 0.001$) with carcass P retention. The ratio of carcass Ca retention to carcass P retention in the current study ranged from 1.06 to 1.30, depending on the dietary treatment, which is lower than the bone Ca:P ratio of 2:1. Ostensibly, these findings indicate that 35 to 47% of carcass P was not derived from bones and that a significant portion of dietary P is retained in body tissues.

For carcass P retention, although the linear SID Ca effect was not significant in the full RSM model, it was included in the reduced model as the interaction between SID Ca and SID P was significant.

Overall Conclusions

Several general observations were made from the 3 studies of predicting the SID Ca requirements of broilers from hatch to market age. The first is that the predicted requirements for maximum tibia ash were higher than those for maximum weight gain in broiler starters and growers, but similar in finishers. Higher digestible Ca requirements for maximum bone ash than for weight gain have also been reported in growing pigs (González-Vega et al., 2016a,b; Lagos et al., 2019a,b). The unresolved question, therefore, is which criteria (weight gain or tibia ash) should be used when recommending digestible Ca requirements. Clearly, Ca requirement should not only represent the minimum amount of Ca required to produce the best growth but also support good skeletal health and prevent welfare issues.

Data on digestible Ca recommendations for poultry are scant. Only one other study is available for comparison with the current work. Walk et al. (2021; 2022a,b) recommended SID Ca requirements for the maximum bone ash in 0 to 10, 11 to 24 and 25 to 42-dayold broilers as 5.30, 5.15, and 3.70 g/kg, respectively. Corresponding values in our studies (David et al., 2021c; 2022; current work) were lower in 1 to 10, 11 to 24, and 25 to 35-day-old broilers at 4.51, 3.69, and 3.00 to 3.50 g/kg, respectively. Possible reasons for these differences were previously discussed (David et al., 2022).

Second, though the digestible Ca requirement could not be predicted for broiler finishers in the present study, the general trend suggests that the requirement declined with advancing broiler age. This finding is unsurprising, since the demand for Ca for bone growth is higher in young birds and declines as the birds grow older (Ravindran and Abdollahi, 2021).

Third, the linear Ca effect on tibia ash observed in the current study prevented the prediction of digestible Ca requirement of the finishers. This observation implied that the SID Ca responses depend on dietary SID P concentrations and that the higher the SID P, the higher will be the requirement of SID Ca. It is, therefore, self-evident that the question of Ca requirement cannot be addressed as a subject distinct from that of P.

DISCLOSURES

There is no conflict of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2023.102492.

REFERENCES

Anwar, M. N. 2017. Measurement of true ileal calcium digestibility of feed ingredients for broiler chickens. PhD Diss. Massey Univ., Palmerston North, New Zealand.

Anwar, M. N., V. Ravindran, P. C. H. Morel, G. Ravindran, and A. J. Cowieson. 2015. Measurement of true ileal calcium digestibility in meat and bone meal for broiler chickens. Anim. Feed Sci. Technol. 206:100–107.

Anwar, M. N., V. Ravindran, P. C. H. Morel, G. Ravindran, and A. J. Cowieson. 2016a. Effect of limestone particle size and calcium to non-phytate phosphorus ratio on true ileal calcium digestibility of limestone for broiler chickens. Br. Poult. Sci. 57:707–713.

Anwar, M. N., V. Ravindran, P. C. H. Morel, G. Ravindran, and A. J. Cowieson. 2016b. Measurement of true ileal calcium digestibility in meat and bone meal for broiler chickens using direct method. Poult. Sci. 95:70–76.

Anwar, M. N., V. Ravindran, P. C. H. Morel, G. Ravindran, and A. J. Cowieson. 2016c. Apparent ileal digestibility of calcium in limestone for broiler chickens. Anim. Feed Sci. Technol. 213:142–147.

Anwar, M. N., V. Ravindran, P. C. H. Morel, G. Ravindran, and A. J. Cowieson. 2017. Effect of calcium source and particle size on the true ileal digestibility and total tract retention of calcium in broiler chickens. Anim. Feed Sci. Technol. 224:39–45.

Anwar, M. N., V. Ravindran, P. C. H. Morel, G. Ravindran, and A. J. Cowieson. 2018. Measurement of the true ileal calcium digestibility of some feed ingredients for broiler chickens. Anim. Feed Sci. Technol. 237:118–128.

AOAC International. 2003. Official Methods of Analysis. 17th ed. Association of Official Analytical Chemists, Washington, DC.

AOAC International. 2016. Official Methods of Analysis. 20th ed. Association of Official Analytical Chemists, Washington, DC.

Browning, L. C., and A. J. Cowieson. 2014. The concentration of strontium and other minerals in animal feed ingredients. J. Appl. Anim. Nutr. 2:1–6 e7.

Caldas, J. V., N. Boonsinchai, J. Wang, J. A. England, and C. N. Coon. 2019. The dynamics of body composition and body energy content in broilers. Poult. Sci. 98:866–877.

Caldwell, R. A. 1992. Effect of calcium and phytic acid on the activation of trypsinogen and the stability of trypsin. J. Agric. Food Chem. 40:43–46.

David, L. S., M. R. Abdollahi, G. Ravindran, C. L. Walk, and V. Ravindran. 2019. Studies on the measurement of ileal calcium digestibility of calcium sources in broiler chickens. Poult. Sci. 98:5582–5589.

David, L. S., M. R. Abdollahi, M. R. Bedford, and V. Ravindran. 2020. Effect of age and dietary crude protein on the apparent ileal calcium digestibility of limestone in broiler chickens. Anim. Feed Sci. Technol. 263:114468.

David, L. S., M. R. Abdollahi, M. R. Bedford, and V. Ravindran. 2021a. True ileal calcium digestibility in soybean meal and canola meal, and true ileal phosphorous digestibility in maize-soybean meal and maize-canola meal diets, without and with microbial phytase, for broiler growers and finishers. Br. Poult. Sci. 62:293–303.

David, L. S., M. R. Abdollahi, M. R. Bedford, and V. Ravindran. 2021b. Comparison of the apparent ileal calcium digestibility of limestone in broilers and layers. Br. Poult. Sci. 62:852–857.

David, L. S., M. R. Abdollahi, M. R. Bedford, and V. Ravindran. 2021c. Requirement of digestible calcium at different dietary concentrations of digestible phosphorus for broiler chickens. 1. Broiler starters (d 1 to 10 post-hatch). Poult. Sci. 100:101439.

David, L. S., M. R. Abdollahi, M. R. Bedford, and V. Ravindran. 2022. Requirement of digestible calcium at different dietary concentrations of digestible phosphorus for broiler chickens. 1. Broiler growers (d 11 to 24 post-hatch). Poult. Sci. 101:102135.

González-Vega, J. C., Y. Liu, J. C. McCann, C. L. Walk, J. J. Loor, and H. H. Stein. 2016a. Requirement for digestible calcium by eleven-to twenty-five-kilogram pigs as determined by growth performance, bone ash concentration, calcium and phosphorous balances, and expression of genes involved in transport of calcium in intestinal and kidney cells. J. Anim. Sci. 94:3321–3334.

González-Vega, J. C., C. L. Walk, M. R. Murphy, and H. H. Stein. 2016b. Requirement for digestible calcium by 25 to 50 kg pigs at different dietary concentrations of phosphorous as indicated by growth performance, bone ash concentration, and calcium and phosphorous balances. J. Anim. Sci. 94:5272–5285.

Lagos, L. V., C. L. Walk, M. R. Murphy, and H. H. Stein. 2019a. Effects of dietary digestible calcium on growth performance and bone ash concentration in 50- to 85-kg growing pigs fed diets with different concentrations of digestible phosphorus. Anim. Feed Sci. Technol. 247:262–272.

Lagos, L. V., S. A. Lee, G. Fondevila, C. L. Walk, M. R. Murphy, J. J. Loor, and H. H. Stein. 2019b. Influence of the concentration of dietary digestible calcium on growth performance, bone mineralization, plasma calcium, and abundance of genes involved in intestinal absorption of calcium in pigs from 11 to 22 kg fed diets with different concentrations of digestible phosphorus. J. Anim. Sci. Biotechnol. 10:47.

Mutucumarana, R. K., and V. Ravindran. 2016. Measurement of true ileal phosphorus digestibility in meat and bone meal for broiler chickens using the direct method. Anim. Feed Sci. Technol. 219:249–256.

Mutucumarana, R. K., and V. Ravindran. 2020. Measurement of endogenous phosphorous losses in broiler chickens: Research note. J. Poult. Sci. 58:58–63.

Mutucumarana, R. K., V. Ravindran, G. Ravindran, and A. J. Cowieson. 2014a. Influence of dietary calcium concentration on the digestion of nutrients along the intestinal tract of broiler chickens. J. Poult. Sci. 51:392–401.

Mutucumarana, R. K., V. Ravindran, G. Ravindran, and A. J. Cowieson. 2014b. Measurement of true ileal digestibility and total tract retention of phosphorous in corn and canola meal for broiler chickens. Poult. Sci. 93:412–419.

Mutucumarana, R. K., V. Ravindran, G. Ravindran, and A. J. Cowieson. 2014c. Measurement of true ileal digestibility of phosphorous in some feed ingredients for broiler chickens. J. Anim. Sci. 92:5520–5529.

Mutucumarana, R. K., V. Ravindran, G. Ravindran, and A. J. Cowieson. 2015a. Measurement of true ileal phosphorous digestibility in maize and soybean meal for broiler chickens: comparison of two methodologies. Anim. Feed Sci. Technol. 206:76–86.

Mutucumarana, R. K., V. Ravindran, G. Ravindran, and A. J. Cowieson. 2015b. Measurement of true ileal phosphorous digestibility in meat and bone meal for broiler chickens. Poult. Sci. 94:1611–1618.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

National Research Council. 2012. Nutrient Requirements of Swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Ravindran, V., and M. R. Abdollahi. 2021. Nutrition and digestive physiology of the broiler chick: state of the art and outlook. Animals 11:2795.

Ravindran, V., L. I. Hew, G. Ravindran, and W. L. Bryden. 1999. A comparison of ileal digesta and excreta analysis for the determination of amino acid digestibility in food ingredients for poultry. Br. Poult. Sci. 40:266–274.

Ravindran, V., L. I. Hew, G. Ravindran, and W. L. Bryden. 2005. Apparent ileal digestibility of amino acids in feed ingredients for broiler chickens. Anim. Sci. 81:85–97.

Ross. 2019. Ross 308 Broiler: Nutrition Specification. Ross Breeders Limited, Newbridge, Midlothian, Scotland, UK.

SAS Institute Inc. 2019. System Requirements for SAS® 9.4 Foundation for Microsoft Windows for x64. SAS Institute Inc, Cary, NC.

Selle, P., A. J. Cowieson, and V. Ravindran. 2009. Calcium interactions with phytate and phytase: consequences for pig and poultry nutrition. Livest. Sci. 124:126–141.

Shastak, Y., M. Witzig, K. Hartung, and M. Rodehutscord. 2012. Comparison of retention and prececal digestibility measurements in evaluating mineral phosphorus sources in broilers. Poult. Sci. 91:2201–2209.

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59:215–221.

van Harn, J., J. W. Spek, C. A. Van Vuure, and M. M. van Krimpen. 2017. Determination of pre-cecal phosphorus digestibility of inorganic phosphates and bone meal products in broilers. Poult. Sci. 96:1334–1340.

Walk, C. L., Z. Wang, S. Wang, J. Wu, J. O. B. Sorbara, and J. Zhang. 2021. Determination of the standardized ileal digestible calcium requirement of male Arbor Acres Plus broilers from hatch to day 10 post-hatch. Poult. Sci. 100:101364.

Walk, C. L., Z. Wang, S. Wang, J. O. B. Sorbara, and J. Zhang. 2022a. Determination of the standardized ileal digestible calcium requirement of male Arbor Acres Plus broilers from day 11 to 24 post-hatch. Poult. Sci. 101:101836.

Walk, C. L., Z. Wang, S. Wang, J. O. B. Sorbara, and J. Zhang. 2022b. Determination of the standardized ileal digestible calcium requirement of male Arbor Acres Plus broilers from day 25 to 42 post-hatch. Poult. Sci. 101:102146.

WPSA. 2013. Determination of phosphorus availability in poultry. Working Group No. 2: Nutrition of the European Federation of Branches of WPSA. Worlds Poult. Sci. J. 69:687–698.

Xue, P. C., K. M. Ajuwon, and O. Adeola. 2016. Phosphorus and nitrogen utilization responses of broiler chickens to dietary crude protein and phosphorus levels. Poult. Sci. 95:2615–2623.