Requirement of digestible calcium at different dietary concentrations of digestible phosphorus for broiler chickens. 2. Broiler growers (d 11 to 24 post-hatch)

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ABSTRACT An experiment was conducted to determine the digestible calcium (**Ca**) and digestible phosphorous (**P**) requirements of 11 to 24 d old broiler chickens. Eighteen corn-soybean meal-based diets containing 1.80, 2.35, 2.90, 3.45, 4.00, and 4.55 g/kg standardized ileal digestible (**SID**) Ca and 3.5, 4.5, and 5.5 g/kg SID P were fed to broilers from d 11 to 24. Each experimental diet was randomly allocated to six replicate cages (8 birds per cage). Body weight and feed amount were recorded at the start and end of the experiment and the feed conversion ratio was calculated. On d 24, birds were euthanized to collect ileal digesta, tibia, and carcass for the determination of digestible Ca and P, the concentration of ash, Ca and P in tibia and the retention of Ca and P in the carcass, respectively. Titanium dioxide (5 g/kg) was included in all diets as an indigestible indicator for apparent ileal digestibility measurement. Total excreta output was 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 effect was significant ($P < 0.05$), the parameter estimate for second-order response surface model was determined using General Linear Model procedure of SAS. The weight gain of broiler growers was optimized at the SID P concentration of 3.5 g/kg and SID Ca concentrations between 2.35 and 4.00 g/kg. At 3.5 g/kg SID P concentration, the required SID Ca for maximum weight gain was determined to be 3.05 g/kg, which corresponded to SID Ca to SID P ratios of 0.87. The concentration of SID Ca that maximized tibia ash at 3.5 g/kg SID P was 3.69 g/kg, which corresponded to SID Ca to SID P ratio of 1.05. Maximizing bone ash requires more Ca than maximizing weight gain. Carcass Ca and P retention were reflective of total tract Ca and P retention values. The estimated SID Ca requirements (at 3.5 g/kg SID P) for both maximized weight gain (3.05 g/kg or 6.11 g/kg total Ca) and bone ash (3.69 g/kg or 7.28 g/kg total Ca) are lower than the current Ca recommendation (8.70 g/kg total Ca equivalent to 4.03 g/kg SID Ca; Ross, 2019) for broiler growers, indicating possible oversupply of Ca in diets formulated based on the current recommendation.

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

2022 Poultry Science 101:102135
https://doi.org/10.1016/j.psj.2022.102135

INTRODUCTION

Currently broiler diets are formulated on the basis of total calcium (**Ca**) and available phosphorus (**aP**), with a ratio of 2:1 being maintained between Ca and aP. There is increasing interest in shifting feed formulations based on digestible Ca due to the recent move towards the use of digestible phosphorous (**P**). Because the absorption and post-absorptive utilization of P and Ca are interrelated, the question of Ca requirement cannot be addressed as a subject distinct from P requirement. To enable this move, as the first step, data on the ileal digestibility of Ca in Ca sources are required.

Limestone is the major inorganic Ca source in broiler diets, supplying up to 70% (Gilani et al., 2022) of the total Ca requirement. Historically it is universally assumed that the Ca from limestone is highly available, but current evidence demonstrates that it is not the case (Walk et al., 2021a). Recent data on the ileal digestibility of Ca in limestone for broilers range between 0.45 and 0.61 (Anwar et al., 2016a,c, 2017; Zhang and Adeola, 2018; David et al., 2019, 2020, 2021b). Several other Ca sources supply the remaining 30% of dietary

Received May 22, 2022.
Accepted August 6, 2022.

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Ca and, these include inorganic phosphates (dicalcium phosphate, monocalcium phosphate), meat and bone meal, and plant-based protein supplements (soybean meal, canola meal). Calcium digestibility of these sources has also become available (Anwar et al., 2015, 2016b, 2018; Zhang and Adeola, 2018; David et al., 2019, 2021a) enabling the initiation of studies on the requirements of digestible Ca and digestible P.

David et al. (2021c) investigated the standardized ileal digestible (**SID**) Ca and SID P requirements in broiler starters (d 1−10 post-hatch) for growth, bone mineralization and mineral utilization. The results showed that the growth performance, bone mineralization, and Ca and P utilization were optimized at 5 g/kg SID P concentration. The SID Ca required for maximum weight gain and tibia ash was determined to be 3.32 and 4.51 g/kg, respectively, at 5.0 g/kg SID P, which corresponded to SID Ca to SID P ratios of 0.66 and 0.90, respectively. The current study, the second in the series, focuses on the determination of requirements of SID Ca and SID P in broiler growers (d 11−24 post-hatch) to maximize growth performance, bone mineralization, and Ca and P retention.

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 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 growers (11−24 d post-hatch) are 8.70 and 4.35 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 4.03 and 4.83 g/kg, respectively. Therefore, a range of SID Ca (1.80−4.55 g/kg) and SID P (3.5 to 5.5 g/kg) which are below and above the recommended values were considered in the development of treatments. For the SID Ca, the selected values were mostly below the target value based on previous findings (David et al., 2021c). The ingredient and calculated nutrient composition of broiler starter crumbles (4.4 g/kg SID Ca; 5.0 g/kg SID P) offered to the broilers from d 1 to 10 are shown in Table 3. Eighteen experimental grower diets based on corn-soybean meal were formulated in a 6 × 3 factorial arrangement with diets containing 6 concentrations of SID Ca and three concentrations of SID P (Table 4). Diets were formulated to contain 1.80, 2.35, 2.90, 3.45, 4.00, and 4.55 g/kg SID Ca (corresponding to 3.84, 4.84, 5.84, 6.84, 7.84, and 8.84 g/kg total Ca, respectively), and 3.5, 4.5 and 5.5 g/kg SID P

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	250	67[4]	168

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

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

Ingredient	Amount
Corn	560
Soybean meal	363
Dicalcium phosphate	10.10
Monosodium phosphate	6.70
Limestone	12.20
Sodium chloride	0.7
Sodium bicarbonate	1.0
DL Methionine	3.8
Lysine HCl	4.8
L Threonine	2.7
L Valine	1.3
Vitamin premix[1,2]	1.0
Mineral premix[1,2]	1.0
Choline chloride 60%	0.8
Soybean oil	31.1
Calculated composition	
SID Ca	4.4
SID P	5.0
SID Ca: SID P	0.89
Total Ca	9.0
Total P	6.80
Non-phytate P	4.88
Total Ca: Non-phytate P	1.85

Abbreviations: Ca, calcium; P, phosphorous; SID, standardized ileal digestible.
[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.

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

SID Ca	1.80			2.35			2.90			3.45			4.00			4.55		
SID P	3.5	4.5	5.5	3.5	4.5	5.5	3.5	4.5	5.5	3.5	4.5	5.5	3.5	4.5	5.5	3.5	4.5	5.5
SID Ca: SID P	0.51	0.40	0.33	0.67	0.52	0.43	0.83	0.65	0.53	0.99	0.77	0.63	1.14	0.89	0.73	1.30	1.01	0.83
Total Ca	3.84	3.84	3.84	4.84	4.84	4.84	5.84	5.84	5.84	6.84	6.84	6.84	7.84	7.84	7.84	8.84	8.84	8.84
Corn	622	618	606	617	613	601	612	609	596	607	604	591	603	599	586	598	594	582
Soybean meal	324	324	326	324	325	327	325	326	328	326	327	328	327	327	329	328	328	330
Dicalcium phosphate	5.94	5.94	5.94	5.96	5.96	5.96	5.99	5.99	5.99	6.01	6.01	6.01	6.03	6.03	6.03	6.06	6.06	6.06
Monosodium phosphate	0.00	5.98	12.01	0.00	5.99	12.01	0.00	5.98	12.00	0.00	5.99	12.01	0.00	5.99	12.01	0.00	5.98	12.01
Limestone	2.77	2.77	2.77	5.20	5.20	5.20	7.62	7.62	7.62	10.04	10.04	10.04	12.47	12.47	12.47	14.89	14.89	14.89
Sodium chloride	0.83	0.84	0.86	0.84	0.85	0.87	0.85	0.86	0.88	0.86	0.87	0.89	0.87	0.88	0.90	0.88	0.88	0.91
Sodium bicarbonate	4.08	0.00	0.00	4.08	0.00	0.00	4.07	0.00	0.00	4.07	0.00	0.00	4.06	0.00	0.00	4.06	0.00	0.00
DL Methionine	3.21	3.22	3.24	3.22	3.22	3.24	3.23	3.23	3.25	3.23	3.24	3.26	3.24	3.25	3.27	3.25	3.26	3.27
Lysine HCl	4.11	4.10	4.07	4.10	4.09	4.06	4.09	4.08	4.05	4.08	4.07	4.04	4.06	4.05	4.02	4.05	4.04	4.01
L Threonine	2.15	2.15	2.15	2.15	2.15	2.15	2.15	2.15	2.15	2.15	2.15	2.15	2.15	2.15	2.15	2.15	2.15	2.15
L Valine	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.95
Vitamin premix[1,2]	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	1.0	1.0	1.0
Trace mineral premix[2]	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	1.0	1.0	1.0
Choline chloride 60%	0.73	0.73	0.74	0.73	0.73	0.74	0.73	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	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	5.0	5.0	5.0
Soybean oil	22.5	23.8	28.0	24.2	25.5	29.7	25.9	27.2	31.4	27.6	28.9	33.1	29.3	30.6	34.8	31.0	32.3	36.5

Abbreviations: SID Ca, standardized ileal digestible calcium; SID P, standardized ileal digestible phosphorous.

[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 pentothenate, 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 trace mineral premixes contained no calcium.

(corresponding to 4.7, 6.2 and 7.7 g/kg total P, respectively) as indicated in Table 5. Concentration of total Ca ranged from 0.44 to 1.02 times the requirement for total Ca (8.70 g/kg; Ross, 2019). All experimental diets were isoenergetic and isonitrogenous. Each diet was separately mixed, steam-conditioned to 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

A total of 864, day-old male broilers (Ross 308) were obtained from a commercial hatchery and raised on floor pens up to d 10. The birds were fed common broiler starter crumbles until d 10. The Ca and P concentrations of this diet were based on the findings of the previous study for starters (David et al., 2021c). On d 11, the birds were weighed and randomly allocated (mean ± SD, 383 ± 11.2 g) to 108 grower cages (8 birds per cage). Each of the 18 experimental diets was offered ad libitum to 6 replicate cages of broilers from d 11 to 24 posthatch. The birds had free access to water.

Measurements

Growth Performance Body weight and feed amount were recorded on a cage basis at the start and end of the experimental period. 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 24, six birds per replicate 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 20, 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 was also recorded during last 4 d to measure the total tract retention of Ca and P.

Bone Mineralization 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 Retention of Ca and P At the start of the trial (d 11), 10 additional chicks were randomly selected and killed by cervical dislocation. At the termination of experiment (d 24), 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.

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

SID Ca	1.80			2.35			2.90			3.45			4.00			4.55		
SID P	3.5	4.5	5.5	3.5	4.5	5.5	3.5	4.5	5.5	3.5	4.5	5.5	3.5	4.5	5.5	3.5	4.5	5.5
SID Ca: SID P	0.51	0.40	0.33	0.67	0.52	0.43	0.83	0.65	0.53	0.99	0.77	0.63	1.14	0.89	0.73	1.30	1.01	0.83
Total Ca	3.84	3.84	3.84	4.84	4.84	4.84	5.84	5.84	5.84	6.84	6.84	6.84	7.84	7.84	7.84	8.84	8.84	8.84
Non-phytate P	2.67	4.16	5.66	2.67	4.16	5.67	2.67	4.17	5.67	2.68	4.17	5.67	2.68	4.18	5.68	2.68	4.18	5.68
Total Ca: Non-phytate P	1.43	0.92	0.68	1.81	1.16	0.85	2.19	1.40	1.03	2.55	1.64	1.21	2.93	1.88	1.38	3.30	2.12	1.56
Dry matter	892	891	888	891	890	887	890	889	885	888	887	884	887	886	883	886	885	881
AME (kcal/kg)	3,050	3,050	3,050	3,050	3,050	3,050	3,050	3,050	3,050	3,050	3,050	3,050	3,050	3,050	3,050	3,050	3,050	3,050
Crude protein	205	205	205	205	205	205	205	205	205	205	205	205	205	205	205	205	205	205
Digestible protein	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166
Starch	389	386	379	386	383	376	383	380	373	380	377	370	377	374	367	374	371	364
Crude fat	45.8	46.9	50.3	47.2	48.3	51.7	48.6	49.6	53.1	50.0	51.0	54.5	51.3	52.4	55.9	52.7	53.8	57.3
Crude fiber	27.9	27.8	27.6	27.8	27.7	27.5	27.7	27.7	27.5	27.6	27.6	27.4	27.6	27.5	27.3	27.5	27.4	27.2
SID Ca	1.80	1.80	1.80	2.35	2.35	2.35	2.90	2.90	2.90	3.45	3.45	3.45	4.00	4.00	4.00	4.55	4.55	4.55
Total P	4.70	6.19	7.68	4.70	6.19	7.68	4.70	6.19	7.68	4.70	6.19	7.68	4.70	6.19	7.68	4.70	6.19	7.68
Phytate P	2.03	2.03	2.02	2.03	2.03	2.02	2.03	2.02	2.01	2.02	2.02	2.01	2.02	2.01	2.00	2.01	2.01	2.00
SID P	3.50	4.50	5.50	3.50	4.50	5.50	3.50	4.50	5.50	3.50	4.50	5.50	3.50	4.50	5.50	3.50	4.50	5.50
Chloride	1.90	1.90	1.90	1.90	1.90	1.90	1.90	1.90	1.90	1.90	1.90	1.90	1.90	1.90	1.90	1.90	1.90	1.90
Sodium	1.90	1.97	3.16	1.90	1.97	3.16	1.90	1.97	3.16	1.90	1.98	3.16	1.90	1.98	3.16	1.90	1.97	3.16
Potassium	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3
Choline (mg/kg)	1,600	1,600	1,600	1,600	1,600	1,600	1,600	1,600	1,600	1,600	1,600	1,600	1,600	1,600	1,600	1,600	1,600	1,600
Dig. threonine	7.70	7.70	7.70	7.70	7.70	7.70	7.70	7.70	7.70	7.70	7.70	7.70	7.70	7.70	7.70	7.70	7.70	7.70
Dig. alanine	7.83	7.82	7.79	7.81	7.81	7.78	7.80	7.79	7.76	7.79	7.78	7.75	7.78	7.77	7.74	7.77	7.76	7.73
Dig. valine	8.70	8.70	8.70	8.70	8.70	8.70	8.70	8.70	8.70	8.70	8.70	8.70	8.70	8.70	8.70	8.70	8.70	8.70
Dig. isoleucine	6.78	6.78	6.79	6.78	6.79	6.79	6.79	6.79	6.80	6.79	6.79	6.80	6.79	6.79	6.80	6.80	6.80	6.80
Dig. leucine	14.4	14.4	14.3	14.4	14.4	14.3	14.4	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.2
Dig. lysine	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5
Dig. arginine	11.6	11.6	11.7	11.6	11.7	11.7	11.7	11.7	11.7	11.7	11.6	11.7	11.7	11.7	11.7	11.7	11.7	11.7
Dig. cysteine	2.83	2.83	2.82	2.83	2.83	2.81	2.82	2.82	2.81	2.82	2.82	2.80	2.82	2.81	2.80	2.81	2.81	2.79
Dig. methionine	5.87	5.87	5.88	5.87	5.88	5.89	5.88	5.88	5.89	5.88	5.88	5.90	5.89	5.89	5.90	5.89	5.89	5.91
Dig. met. + cysteine	8.70	8.70	8.70	8.70	8.70	8.70	8.70	8.70	8.70	8.70	8.70	8.70	8.70	8.70	8.70	8.70	8.70	8.70
Analyzed values[1]																		
Dry matter	899	886	894	898	888	900	896	900	896	903	872	901	900	900	899	894	897	896
Total Ca	3.98	3.86	4.02	4.73	4.86	4.60	5.77	6.56	6.43	6.92	7.26	7.48	8.49	9.02	8.42	9.36	9.71	8.95
Total P	5.24	6.50	8.32	5.08	6.55	7.74	5.11	6.67	8.08	5.51	7.00	8.42	4.83	6.35	7.99	5.78	6.58	8.07

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

Chemical Analysis

Ingredients were analyzed for dry matter (**DM**, method 930.15; AOAC, 2016), ash (method 942.05; AOAC, 2016), nitrogen (968.06; AOAC, 2016), fat (AOAC 2003.06), crude fiber (AOAC 2003.04), Ca and total P (method 968.08D; AOAC, 2016), and phytate P (Caldwell, 1992). The concentrations of ash (AOAC 942.05; AOAC, 2016), Ca and P (AOAC 968.08D; AOAC, 2016) of tibia were determined using standard procedures. The diet, ileal digesta, and excreta samples were analyzed for DM (method 930.15; AOAC, 2016), Ca, total P (method 968.08D; AOAC, 2016), and titanium dioxide (Short et al., 1996). Subsamples of the minced carcass were analyzed for DM (method 930.15; AOAC, 2016), Ca, and P (AOAC 968.08D; AOAC, 2016).

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.

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

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:

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

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:

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

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

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:

$$\text{Retained Ca or P} = \left[(Mc \times \text{CW})_{\text{D24}} - (Mc \times \text{CW})_{\text{D11}}\right]$$

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 D24 and D11 denote 24-day-old and 11-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 $P \leq 0.05$ and the tendency was considered at $P \leq 0.10$. Second, if the interaction or main effect was significant, then the parameter estimate for the second-order response surface model 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. (2016). 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:

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

where SID Ca $_{\max}$ is the concentration of SID Ca at the maximum 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 relationships between measured parameters were analyzed by Pearson Correlations (SAS, 2019).

RESULTS

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

Determined concentrations of SID Ca and SID P of the 18 assay diets, in comparison with formulated values, are summarized in Table 7. The difference between calculated and determined SID Ca concentrations of the experimental diets was comparable, except in 6 of 18 diets. The percent difference between calculated and determined SID Ca concentrations in these 6 diets was more than 20% and ranged from 24 to 33%. The determined SID Ca values were higher than the calculated SID Ca values when the dietary SID Ca was at or below 2.90 g/kg (1.80−2.90 g/kg) whereas the determined SID Ca values were lower than the calculated SID Ca values when the dietary SID Ca was above 2.90 g/kg (3.45 −4.55 g/kg). The calculated and determined SID Ca values were comparable at 3.5 g/kg SID P and 2.35 to 3.45 g/kg SID Ca concentrations. 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 23%. The determined SID P values were, however, lower than the calculated SID P values when the diets contained SID Ca of 3.45 g/kg and above.

Growth Performance

The weight gain, feed intake and FCR of broilers fed diets containing different concentrations of SID Ca and SID P from d 11 to 24 are summarized in Table 8 and the trends are illustrated in Figure 1. There was an interaction ($P < 0.001$) between SID Ca and SID P for the weight gain and feed intake. At the lowest SID Ca (1.80 g/kg) concentration, increasing concentration of SID P reduced the weight gain and feed intake. However, increasing SID P concentration increased the weight

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	930
Ash	12	65	996	848	-
Crude protein	75	475	-	-	-
Fat	39	12	-	-	1.0
Neutral detergent fiber	88	86	-	-	-
Calcium	0.08	3.40	410	260	-
Total P	2.20	6.90	0.56	185	250
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	182

[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.

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

SID Ca	1.80			2.35			2.90			3.45			4.00			4.55		
SID P	3.5	4.5	5.5	3.5	4.5	5.5	3.5	4.5	5.5	3.5	4.5	5.5	3.5	4.5	5.5	3.5	4.5	5.5
Determined SID Ca	2.39	2.23	2.40	2.37	2.37	2.34	2.98	3.38	3.19	3.47	2.55	3.01	3.69	3.84	3.76	3.95	3.48	3.37
Determined SID P	3.68	4.78	6.26	3.37	4.50	5.88	3.06	4.38	5.59	3.15	3.90	5.45	2.69	3.66	5.06	2.91	3.53	4.79
Determined SID Ca: SID P	0.65	0.47	0.38	0.70	0.53	0.40	0.97	0.77	0.57	1.10	0.65	0.55	1.37	1.05	0.74	1.36	0.99	0.70
Difference (calculated minus determined)																		
SID Ca	-0.59	-0.43	-0.60	-0.02	-0.02	0.01	-0.08	-0.48	-0.29	-0.02	0.90	0.44	0.31	0.16	0.24	0.60	1.07	1.18
SID P	-0.18	-0.28	-0.76	0.13	0.00	-0.38	0.44	0.12	-0.09	0.35	0.60	0.05	0.81	0.84	0.44	0.59	0.97	0.71

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

gain and feed intake at the highest SID Ca concentration (4.55 g/kg), but had no effect on the performance at SID Ca concentrations between 2.90 and 4.00 g/kg. A reduced model was used for the prediction of SID Ca at maximum response for weight gain and FCR whereas the full model was used for feed intake. The predicted maximum weight gains at SID P concentrations of 3.5, 4.5, and 5.5 g/kg were 1,174, 1,177, and 1,200 g/bird, at SID Ca concentrations of 3.05, 3.69, and 4.33 g/kg, respectively. These values corresponded to SID Ca to SID P ratios of 0.87, 0.82, and 0.79, respectively. The predicted maximum feed intake at SID P concentrations of 3.5, 4.5, and 5.5 g/kg were 1,514, 1,526, and 1,520 g/bird, at SID Ca concentrations of 2.64, 3.40, and 4.16 g/kg, respectively. These values corresponded to SID Ca to SID P ratios of 0.75, 0.76, and 0.76, respectively. The FCR was influenced by main effect of SID Ca wherein the lowest FCR was observed when birds were fed SID Ca concentration of 3.45 g/kg. The predicted minimum FCR at SID P concentrations of 3.5, 4.5, and 5.5 g/kg were 1.29, 1.29, and 1.27, at SID Ca concentrations of 3.59, 3.99, and 4.39 g/kg, respectively. These values corresponded to SID Ca to SID P ratios of 1.03, 0.89, and 0.80, respectively.

Table 8. Growth performance of broiler chickens fed diets containing different concentration of standardized ileal digestible (SID) calcium (Ca) and SID phosphorous (P) from d 11 to 24[1].

SID Ca	SID P	Weight gain (g/bird)	Feed intake (g/bird)	FCR
1.80	3.5	1,129[bcd]	1,488[ab]	1.32
	4.5	1,082[de]	1,450[bc]	1.35
	5.5	1,043[e]	1,407[c]	1.35
2.35	3.5	1,152[abc]	1,516[a]	1.32
	4.5	1,129[bcd]	1,501[ab]	1.33
	5.5	1,099[cd]	1,447[bc]	1.32
2.90	3.5	1,185[a]	1,537[a]	1.30
	4.5	1,167[ab]	1,519[a]	1.30
	5.5	1,147[abc]	1,480[ab]	1.30
3.45	3.5	1,182[ab]	1,495[ab]	1.28
	4.5	1,177[ab]	1,518[a]	1.29
	5.5	1,161[ab]	1,481[ab]	1.27
4.00	3.5	1,156[ab]	1,485[ab]	1.29
	4.5	1,170[ab]	1,513[a]	1.29
	5.5	1,189[a]	1,528[a]	1.28
4.55	3.5	1,085[de]	1,396[c]	1.31
	4.5	1,191[a]	1,526[a]	1.28
	5.5	1,200[a]	1,516[a]	1.27
SEM[2]		19.9	20.6	0.012
Main effects				
SID Ca				
1.80		1,085	1,448	1.34[a]
2.35		1,127	1,488	1.32[a]
2.90		1,166	1,512	1.30[b]
3.45		1,173	1,498	1.28[c]
4.00		1,172	1,509	1.29[bc]
4.55		1,159	1,479	1.29[bc]
SEM		11.2	11.9	0.007
SID P				
3.5		1,148	1,486	1.30
4.5		1,153	1,505	1.31
5.5		1,140	1,476	1.30
SEM		8.1	8.4	0.005
Probability, $P \leq$				
SID Ca		0.001	0.003	0.001
SID P		0.543	0.062	0.616
SID Ca × SID P		0.001	0.001	0.370

[1]Each value represents the mean of six replicates (eight birds per replicate).

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

[2]Pooled standard error of mean.

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

Data on SIDC of Ca and P, intake of SID Ca and SID P and ratio of SID Ca intake to SID P intake in 11- to 24-day-old broilers are presented in Table 9 and Figure 2. The main effects of SID Ca concentration were significant for the SIDC of Ca and digestible Ca intake. The SIDC of Ca decreased linearly ($P < 0.001$) and SID Ca intake linearly increased ($P < 0.001$) with increasing SID Ca concentration. Therefore, the maximum values were not calculated for these two parameters. Standardized ileal P digestibility and SID P intake were increased ($P < 0.001$) with increasing SID P and decreasing SID Ca concentration. The reduced model was used to predict the minimum values for the SIDC of P. The predicted minimum ileal P digestibility coefficient at SID P concentrations of 3.5, 4.5, and 5.5 g/kg were 0.52, 0.57, and 0.62, at the SID Ca concentration of 6.67 g/kg. These values corresponded to SID Ca to SID P ratios of 1.91, 1.48, and 1.21, respectively. The ratio of SID Ca intake to SID P intake was higher at 3.5 g/kg SID P and decreased with increasing SID P concentration at all SID Ca concentrations. But the magnitude of decreases was greater as the SID Ca concentration increased, resulting in a SID Ca × SID P interaction ($P < 0.05$).

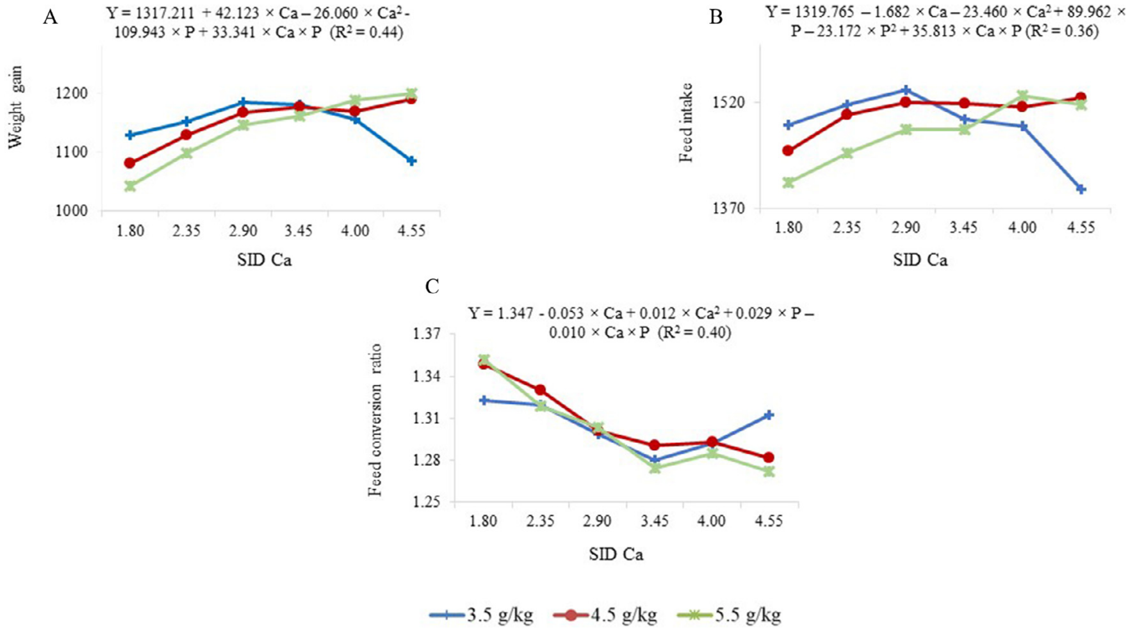

Figure 1. (A) Weight gain (g/bird), (B) feed intake (g/bird), and (C) feed conversion ratio of broiler chickens fed different standardized ileal digestible (SID) calcium (Ca) and SID phosphorous (P) concentration (3.5, 4.5, and 5.5 g/kg) from d 11 to 24.

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 concentration (g/kg) of SID Ca and SID P from d 11 to 24[1].

SID Ca	SID P	SIDC of Ca	SID Ca intake	SIDC of P	SID P intake	SID Ca intake: SID P intake
1.80	3.5	0.67	3.56	0.77	5.47	0.65[ef]
	4.5	0.64	3.24	0.81	6.93	0.47[gh]
	5.5	0.68	3.38	0.86	8.80	0.38[h]
2.35	3.5	0.56	3.60	0.71	5.11	0.70[de]
	4.5	0.56	3.56	0.76	6.75	0.53[g]
	5.5	0.55	3.38	0.80	8.51	0.40[h]
2.90	3.5	0.53	4.60	0.65	4.72	0.97[c]
	4.5	0.60	5.13	0.73	6.65	0.77[d]
	5.5	0.57	4.71	0.77	8.28	0.57[fg]
3.45	3.5	0.53	5.18	0.66	4.70	1.10[b]
	4.5	0.40	3.86	0.67	5.91	0.65[ef]
	5.5	0.46	4.46	0.74	8.08	0.55[fg]
4.00	3.5	0.47	5.47	0.56	4.00	1.37[a]
	4.5	0.49	5.81	0.61	5.53	1.04[bc]
	5.5	0.48	5.73	0.69	7.72	0.74[de]
4.55	3.5	0.47	5.45	0.61	4.04	1.35[a]
	4.5	0.42	5.32	0.59	5.39	0.99[c]
	5.5	0.40	5.11	0.66	7.27	0.71[de]
SEM[2]		0.028	0.250	0.015	0.147	0.038
Main effects						
SID Ca						
1.80		0.66[a]	3.39[c]	0.82[a]	7.07[a]	0.50
2.35		0.56[b]	3.52[c]	0.76[b]	6.79[b]	0.54
2.90		0.57[b]	4.81[b]	0.72[c]	6.55[c]	0.77
3.45		0.47[cd]	4.50[b]	0.69[c]	6.23[d]	0.77
4.00		0.48[c]	5.67[a]	0.62[d]	5.75[e]	1.05
4.55		0.43[d]	5.29[a]	0.62[d]	5.57[e]	1.01
SEM		0.016	0.144	0.009	0.085	0.022
SID P						
3.5		0.54	4.64	0.66[c]	4.67[c]	1.02
4.5		0.52	4.49	0.70[b]	6.19[b]	0.74
5.5		0.52	4.46	0.75[a]	8.11[a]	0.56
SEM		0.011	0.102	0.006	0.060	0.015
Probability, $P \leq$						
SID Ca		0.001	0.001	0.001	0.001	0.001
SID P		0.408	0.398	0.001	0.001	0.001
SID Ca × SID P		0.104	0.073	0.063	0.357	0.001

[1]Each value represents the mean of six replicates (six birds per replicate).

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

[2]Pooled standard error of mean.

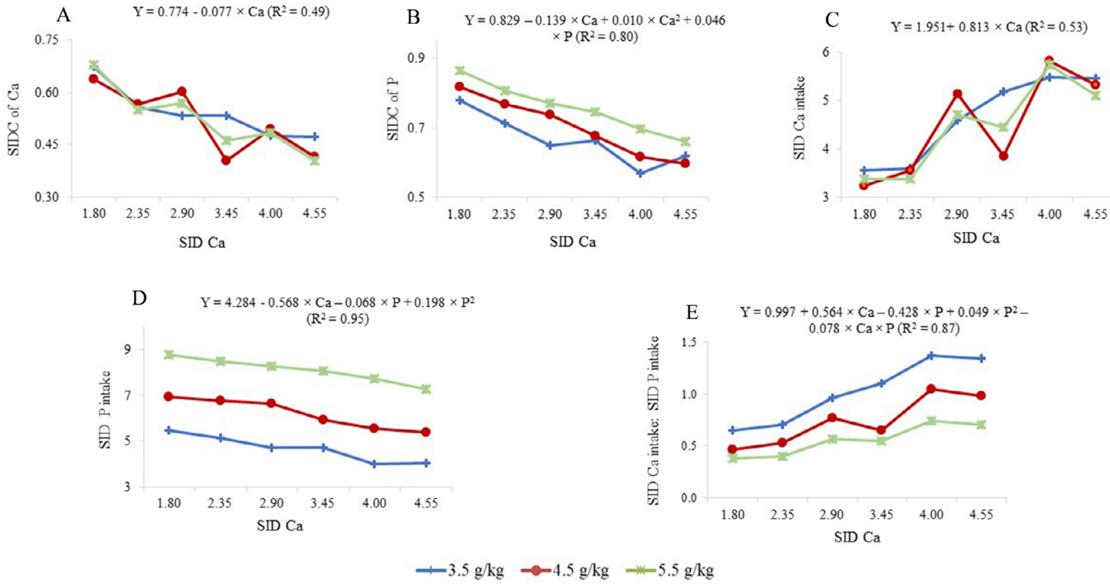

Figure 2. (A) Standardized ileal digestibility coefficients (SIDC) of calcium (Ca) and (B) phosphorous (P); intake (g/bird) of (C) standardized ileal digestible (SID) Ca and (D) SID P; and (E) ratio of SID Ca intake: SID P intake, of broiler chickens fed different concentration of SID Ca and SID P (3.5, 4.5, and 5.5 g/kg) from d 11 to 24.

The maximum values were not calculated for the SID P intake and the ratio of SID Ca intake: SID P intake due to the linear Ca effect.

Bone Mineralization

Table 10 and Figure 3 present the concentrations of ash, Ca, and P of tibia in 11- to 24-day-old broilers fed diets containing different SID Ca and SID P concentrations. Based on factorial arrangement of treatments, there was no interaction ($P > 0.05$) between SID Ca and SID P for any tibia parameter. Tibia ash was increased ($P < 0.05$) by increasing concentration of both SID Ca and SID P. A reduced model was used to predict the maximum values for tibia parameters. The predicted maximum tibia ash concentration at SID P concentrations of 3.5, 4.5, and 5.5 g/kg were 401, 413, and 428 g/kg, at SID Ca concentrations of 3.69, 4.30, and 4.91 g/kg, respectively. These values corresponded to SID Ca to SID P ratios of 1.05, 0.96, and 0.89, respectively. Tibia Ca was increased ($P < 0.05$) by increasing SID Ca concentration. The predicted maximum tibia Ca at SID P concentrations of 3.5, 4.5, and 5.5 g/kg were 142, 146, and 150 g/kg, at SID Ca concentrations of 4.06, 4.55, and 5.04 g/kg, respectively. These values corresponded to SID Ca to SID P ratios of 1.16, 1.01, and 0.92, respectively. Tibia P was reduced at the lowest SID Ca (1.80 g/kg) and at the lowest SID P (3.5 g/kg) concentration. Maximum values were not calculated for tibia P due to the linear effects of SID Ca and SID P.

Coefficients of Apparent Total Tract Retention and Retained Ca and P

Table 11 and Figure 4 present the ATTRC and, retained Ca and P in 24-day-old broilers fed diets containing different concentrations of SID Ca and SID P. The ATTRC of Ca decreased ($P < 0.05$) with increasing SID Ca concentration, but was unaffected by SID P concentration. Retained Ca (g/bird) was increased ($P < 0.05$) with increasing SID Ca concentration to 4.00 g/kg and then reduced. The predicted maximum retained Ca (g/bird) at SID P concentrations of 3.5, 4.5, and 5.5 g/kg was 5.09 g/bird, at the SID Ca concentration of 4.39 g/kg. These values corresponded to SID Ca to SID P ratios of 1.25, 0.97, and 0.80, respectively. The maximum value was not estimated for the ATTRC of Ca due to the linear Ca effect.

At all SID Ca concentrations, the ATTRC of P decreased ($P < 0.05$) with increasing SID P concentration. The only exception was at 1.80 g/kg SID Ca, where the ATTRC of P was similar between 4.5 and 5.5 g/kg SID P, resulting in a SID Ca × SID P interaction ($P < 0.05$). Retained P (g/bird) was influenced by neither the main effect of SID P nor the interaction. However, the retained P tended ($P = 0.097$) to be reduced at lower SID Ca concentration. The maximum values were not estimated for the ATTRC of P and retained P due to the linear Ca effect.

The ratio between retained Ca and retained P increased ($P < 0.001$) with increasing SID Ca up to 4.00 g/kg SID Ca and then declined. The ratio was not influenced ($P > 0.05$) by SID P concentration. The predicted maximum ratio of retained Ca to retained P at SID P concentrations of 3.5, 4.5, and 5.5 g/kg was 1.05 at SID Ca concentration of 4.13 g/kg. These values corresponded to SID Ca to SID P ratios of 1.18, 0.92, and 0.75, respectively.

Carcass Retention of Ca and P

Data on retention of Ca and P in the carcass of 24-day-old broilers fed diets containing different SID Ca and SID P concentrations are presented in Table 12 and

Table 10. Concentration of ash, calcium (Ca) and phosphorous (P) in tibia (g/kg dried defatted matter) and toe ash concentration (g/kg, as received basis) in broiler chickens fed diets containing different concentration (g/kg) of standardized ileal digestible (SID) Ca and SID P from d 11 to 24[1].

SID Ca	SID P	Tibia ash	Tibia Ca	Tibia P
1.80	3.5	385	132	70.0
	4.5	385	132	70.6
	5.5	387	130	68.5
2.35	3.5	398	139	72.7
	4.5	400	136	72.5
	5.5	403	139	73.5
2.90	3.5	400	139	72.6
	4.5	405	144	71.9
	5.5	417	144	74.9
3.45	3.5	396	139	68.6
	4.5	403	141	76.6
	5.5	415	144	74.7
4.00	3.5	402	141	72.0
	4.5	420	150	75.7
	5.5	423	145	75.3
4.55	3.5	394	141	68.4
	4.5	420	148	75.1
	5.5	425	149	76.9
SEM[2]		5.2	2.8	1.73
Main effects				
SID Ca				
1.80		386[c]	132[d]	69.7[b]
2.35		400[b]	138[c]	72.9[a]
2.90		408[ab]	142[abc]	73.1[a]
3.45		405[b]	141[bc]	73.3[a]
4.00		415[a]	146[ab]	74.3[a]
4.55		413[a]	146[a]	73.5[a]
SEM[2]		3.0	1.6	1.00
SID P				
3.5		396[c]	138	70.7[b]
4.5		406[b]	142	73.7[a]
5.5		412[a]	142	74.0[a]
SEM[2]		2.1	1.2	0.7
Probability, $P \leq$				
SID Ca		0.001	0.001	0.032
SID P		0.001	0.057	0.002
SID Ca × SID P		0.178	0.506	0.083

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

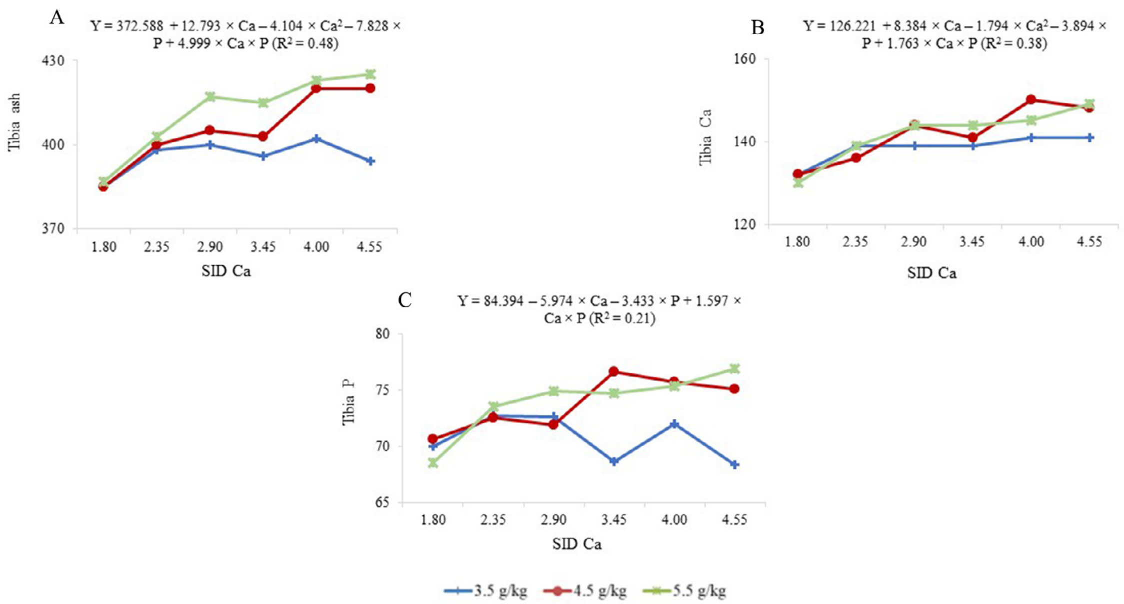

Figure 3. Concentration (g/kg dried defatted matter) of (A) ash, (B) calcium (Ca), and (C) phosphorous (P) of tibia in broiler chickens fed different concentration (g/kg) of standardized ileal digestible (SID) Ca and SID P (3.5, 4.5, and 5.5 g/kg) from d 11 to 24.

Table 11. Apparent total tract retention coefficients (ATTRC) of calcium (Ca) and phosphorous (P) and retained Ca and P (g/bird) in 24-day-old broilers fed diets containing different concentration 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
1.80	3.5	0.64	3.34	0.66[a]	4.65	0.72
	4.5	0.64	3.18	0.46[ef]	3.87	0.83
	5.5	0.63	3.19	0.42[f]	4.24	0.76
2.35	3.5	0.55	3.51	0.66[a]	4.67	0.75
	4.5	0.58	3.75	0.51[cde]	4.51	0.85
	5.5	0.56	3.36	0.44[f]	4.52	0.75
2.90	3.5	0.54	4.49	0.63[ab]	4.48	1.01
	4.5	0.53	4.71	0.52[cd]	4.58	1.06
	5.5	0.54	4.64	0.43[f]	4.84	0.97
3.45	3.5	0.48	4.85	0.64[ab]	4.67	1.05
	4.5	0.43	4.13	0.53[c]	4.78	0.87
	5.5	0.47	4.42	0.43[f]	4.63	0.96
4.00	3.5	0.47	5.43	0.64[a]	4.43	1.23
	4.5	0.47	5.65	0.59[b]	5.38	1.05
	5.5	0.48	5.71	0.44[f]	4.86	1.18
4.55	3.5	0.37	4.77	0.64[a]	4.66	1.02
	4.5	0.39	4.74	0.53[c]	4.75	1.00
	5.5	0.40	5.01	0.47[def]	5.20	0.96
SEM[2]		0.014	0.227	0.019	0.285	0.066
Main effects						
SID Ca						
1.80		0.64[a]	3.23[c]	0.51	4.25	0.77[c]
2.35		0.56[b]	3.54[c]	0.54	4.57	0.78[c]
2.90		0.54[c]	4.61[b]	0.53	4.63	1.01[b]
3.45		0.46[d]	4.47[b]	0.53	4.69	0.96[b]
4.00		0.47[d]	5.59[a]	0.56	4.89	1.15[a]
4.55		0.38[e]	4.84[b]	0.55	4.87	0.99[b]
SEM[2]		0.008	0.131	0.011	0.164	0.038
SID P						
3.5		0.51	4.40	0.65	4.59	0.97
4.5		0.51	4.36	0.52	4.65	0.94
5.5		0.51	4.39	0.44	4.71	0.93
SEM[2]		0.006	0.093	0.008	0.116	0.027
Probability, $P \leq$						
SID Ca		0.001	0.001	0.064	0.097	0.001
SID P		0.697	0.951	0.001	0.763	0.603
SID Ca × SID P		0.355	0.542	0.045	0.322	0.367

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

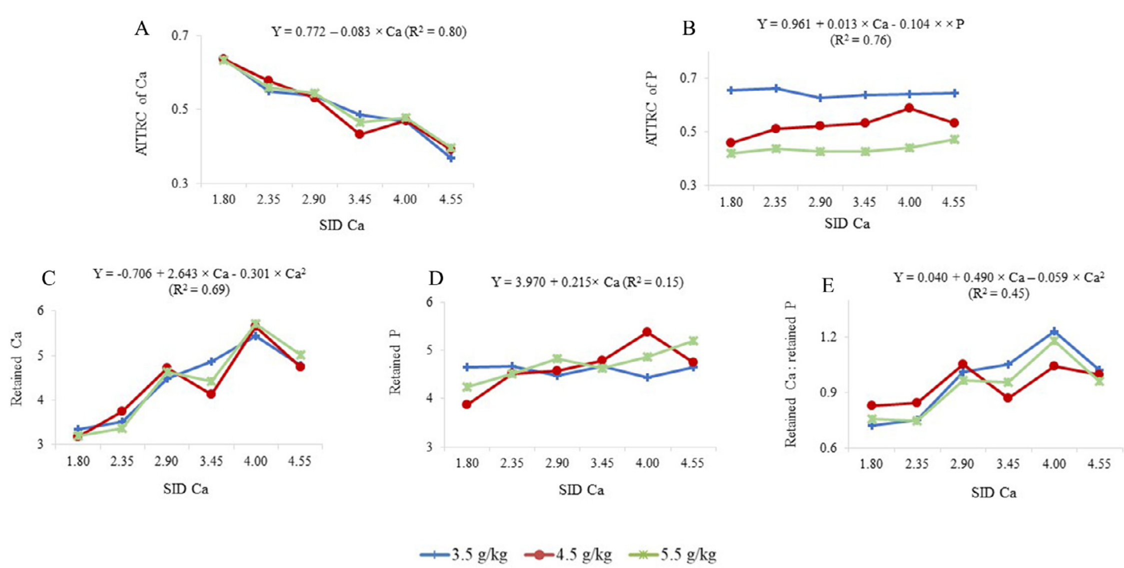

Figure 4. Apparent total tract retention coefficient (ATTRC) of (A) Ca and (B) phosphorous (P); retained (g/bird) (C) Ca and (D) P; (E) ratio of retained Ca to retained P, of broiler chickens fed different concentration (g/kg) of standardized ileal digestible (SID) Ca and SID P (3.5, 4.5, and 5.5 g/kg) from d 11 to 24.

Table 12. Retention (g/bird) of calcium (Ca) and phosphorous (P) in the carcass of 24-day-old broilers fed diets containing different concentration of (g/kg) standardized ileal digestible (SID) Ca and SID P[1,2,3].

SID Ca	SID P	Ca	P
1.80	3.5	4.47[ghij]	4.11[fgh]
	4.5	3.87[jk]	3.77[h]
	5.5	3.47[k]	3.65[h]
2.35	3.5	4.11[ijk]	3.88[gh]
	4.5	4.88[fghi]	4.17[efgh]
	5.5	4.33[hijk]	3.91[gh]
2.90	3.5	5.09[efgh]	4.35[efg]
	4.5	5.38[defg]	4.52[def]
	5.5	6.96[ab]	5.20[a]
3.45	3.5	5.57[def]	4.54[cdef]
	4.5	5.66[cdef]	4.69[abcde]
	5.5	7.01[ab]	5.20[a]
4.00	3.5	5.83[cde]	4.54[cdef]
	4.5	6.52[abc]	5.06[abc]
	5.5	6.58[abc]	4.95[abcd]
4.55	3.5	4.86[fghi]	4.01[fgh]
	4.5	7.11[a]	5.14[ab]
	5.5	6.17[bcd]	4.66[bcde]
SEM[2]		0.331	0.189
Main effects			
SID Ca			
1.80		3.94	3.84
2.35		4.44	3.99
2.90		5.81	4.69
3.45		6.08	4.81
4.00		6.31	4.85
4.55		6.04	4.60
SEM[2]		0.191	0.109
SID P			
3.5		4.99	4.24
4.5		5.57	4.56
5.5		5.75	4.60
SEM[2]		0.135	0.077
Probability, $P \leq$			
SID Ca		0.001	0.001
SID P		0.001	0.002
SID Ca × SID P		0.001	0.002

[1]Each value represents the mean of six replicates (two birds per replicate). The term ‘carcass’ refers to the whole body without feathers.

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

[2]Ca and P retained in the carcass of 11-day-old bird is 1.694 and 1.381 g/bird, respectively, and these values are deducted from the total retained Ca or P at d 24.

[3]Pooled standard error of mean.

Figure 5. A reduced model was used for these parameters. An interaction was observed between SID Ca and SID P for the carcass retention of Ca ($P < 0.001$) and P ($P < 0.01$). At the lowest SID Ca concentration (1.80 g/kg), the carcass Ca retention was higher at lower SID P (3.5 g/kg). However, at 2.90 and 3.45 g/kg SID Ca concentrations, the carcass Ca retention was higher at higher SID P (5.5 g/kg). At 4.55 g/kg SID Ca concentration, the retention was higher at 4.5 g/kg SID P followed by 5.5 g/kg SID P. The predicted maximum carcass Ca retention at SID P concentrations of 3.5, 4.5 and 5.5 g/kg was 5.92, 6.59, and 7.36 g/bird, at SID Ca concentrations of 3.87, 4.20, and 4.53 g/kg, respectively. These values corresponded to SID Ca to SID P ratios of 1.11, 0.93, and 0.82, respectively. At 2.90 and 3.45 g/kg SID Ca concentrations, carcass P retention was higher at 5.5 g/kg SID P whereas at 4.55 g/kg SID Ca, the retention was higher at both 4.5 and 5.5 g/kg SID P. However, no influence of SID P concentration was observed at other SID Ca concentrations. The predicted maximum carcass P retention at SID P concentrations of 3.5, 4.5, and 5.5 g/kg was 4.67, 4.96 and 5.29 g/bird, at SID Ca concentrations of 3.64, 3.96, and 4.27 g/kg, respectively. These values corresponded to SID Ca to SID P ratios of 1.04, 0.88, and 0.78, respectively.

DISCUSSION

In general, analyzed concentrations 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, 2015). It must be pointed out that the same batches of limestone and dicalcium phosphate, used in the broiler starter requirement study (David et al., 2021c), were utilized in the current experiment.

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

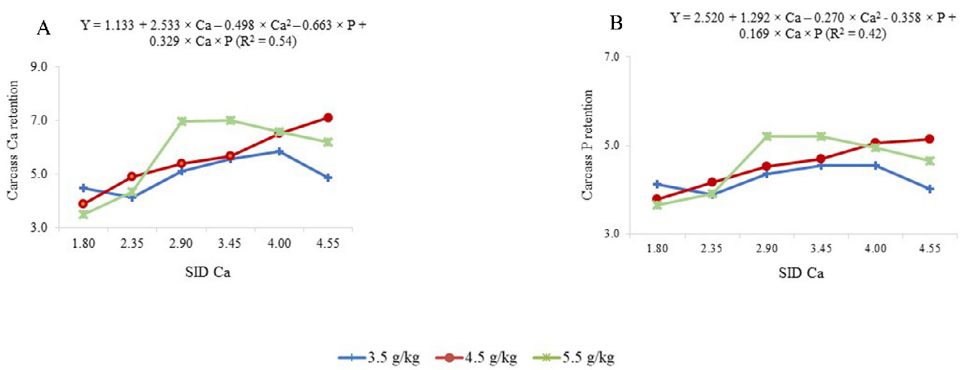

Figure 5. Carcass retention (g/bird) of (A) calcium (Ca), and (B) phosphorous (P) in 24-day-old broiler chickens fed different concentration (g/kg) of standardized ileal digestible (SID) Ca and SID P (3.5, 4.5, and 5.5 g/kg).

ingredients. However, the determined SID Ca values were above the calculated SID Ca when diets were formulated at or below 2.90 g/kg dietary SID Ca, whereas the determined values were below the calculated SID Ca when SID Ca was above 2.90 g/kg. Most likely reason contributing to these discrepancies could be the formation of Ca-P complex in the digestive tract (Selle et al., 2009) and the resultant low Ca digestibility when the dietary Ca is oversupplied (above 2.90 g/kg), which may have resulted in lower determined SID Ca values. On the other hand, at or below 2.90 g/kg SID Ca, the SID Ca values are higher, which could be due to the increased Ca digestibility at lower dietary SID Ca (Table 9). Interestingly, the difference between calculated and determined SID Ca was negligible at 3.5 g/kg SID P when the SID Ca concentration was between 2.35 and 3.45 g/kg, suggesting the relevance of using 3.5 g/kg dietary SID P.

In general, a similar trend was observed for the difference between calculated and determined SID P. At 3.45 g/kg dietary SID Ca and above, the determined SID P values were lower than the calculated SID P especially at lower SID P concentrations (3.5 and 4.5 g/kg), further confirming the formation of Ca-P complex.

Requirements for SID P to Maximize Growth Performance, Bone Mineralization, and Utilization of Ca and P

In the current study, 3 SID P concentrations were used to determine the SID Ca and SID P requirements in 11 to 24-day-old broilers. As stated earlier, a range of SID P (3.5−5.5 g/kg) which is below and above the recommended dietary P concentration (4.35 g/kg aP; Ross, 2019) were considered in the development of dietary treatments. Based on the current data, a SID P concentration of 3.5 g/kg is recommended for broiler growers (11−24 d post-hatch) at SID Ca concentration at or below 4.00 g/kg. The recommendation of SID P for broiler growers (3.5 g/kg) is lower than that determined for broiler starters (5.0 g/kg SID P; David et al., 2021c) demonstrating the age effect on P requirements. Decreasing trends in P digestibility with advancing broiler age have been reported (Fonolla et al., 1981; Shastak et al., 2012) and this may be a reflection of lower P requirements in older birds compared to younger birds. A similar decreasing trend in ileal Ca digestibility with age was reported by David et al. (2020). Létourneau-Montminy et al. (2008), based on a meta-analysis, reported that 3.0 to 3.4 g/kg dietary non-phytate P can be used with reduced dietary Ca (6 g/kg total Ca) to achieve broiler performance similar to that of 4.5 g/kg non-phytate P and 10 g/kg total Ca, proposing reduced P use in broilers. In the same study, however, it was found that the tibia ash concentration in birds fed 6 g/kg dietary total Ca was lower than those fed 10 g/kg Ca. The current findings also showed that the dietary P concentration must be increased if the dietary Ca is increased. Overall, a SID P concentration of 3.5 g/kg is recommended for broiler growers if the SID Ca concentration is 4.00 g/kg or below.

Requirements for SID Ca to Maximize Growth Performance

In the present study, weight gain and feed intake were not influenced by dietary SID P concentration (3.5−5.5 g/kg) when SID Ca concentration was between 2.35 and 4.00 g/kg for weight gain and 2.90 to 4.00 g/kg for feed intake, suggesting the possibility of using lower dietary SID P (3.5 g/kg) within these SID Ca ranges. In addition, weight gains were similar within the range of SID Ca to SID P ratio of 0.53 to 1.14. It must also be noted that 3.5 g/kg SID P increased the growth performance at the lowest SID Ca (1.80 g/kg) concentration. On the other hand, 3.5 g/kg SID P reduced the weight gain and feed intake when the dietary SID Ca was 4.55 g/kg, demonstrating the negative effect of excess dietary Ca which could have bound the P, making it unavailable for absorption and consequently reducing the growth performance. Dietary P is important for body protein deposition and consequent muscle growth (Xue et al., 2016). This negative effect of excess Ca on growth performance was ameliorated as the dietary P concentration was increased to 4.5 and 5.5 g/kg, indicating an increase in dietary SID Ca requires increased dietary SID P to maximize the growth performance. Similar findings were also reported in broiler starters by David et al. (2021c). Considering the optimum performance at a marginal dietary P concentration, it is evident that the SID P concentration of 3.5 g/kg was adequate in broiler grower diets. Based on the response surface analysis, the concentration of SID Ca that maximized weight gain at 3.5 g/kg SID P was 3.05 g/kg (equal to 6.11 g/kg total Ca), which corresponded to a SID Ca to SID P ratio of 0.87. The determined requirements of SID Ca (3.32 g/kg) and SID P (5.0 g/kg) for optimum weight gain of broiler starters (David et al., 2021c) were higher than those determined for broiler growers in the current study (3.05 g/kg SID Ca and 3.5 g/kg SID P). David et al. (2020) reported that the Ca digestibility in limestone decreased with advancing broiler age, which may be due to reduced Ca requirements with age. The findings of Fonolla et al. (1981), Shastak et al. (2012), and Li et al. (2018) are also in agreement with the similar age effect on Ca digestibility. Interestingly, the magnitude of reduction in the requirement of SID Ca and SID P from the starter (David et al. 2021c) to grower (current study) phase are 8.1 and 30.0%, respectively, suggesting that SID P requirement is reduced to a greater extent in broiler growers than that of SID Ca. This may be the reason for the increased SID Ca to SID P ratio requirement for the maximization of weight gain in broiler growers (0.87) when compared to that of broiler starters (0.66). This finding is contrary to the same ratio (2.0) of total Ca to aP recommended by Ross 308 (2019) for all growth phases of broilers.

For both feed intake and FCR, although the linear SID P effect was not significant in the full model, SID P was included in the reduced model as the interaction between SID Ca and SID P was significant. The FCR was similar among the birds fed 3.45 to 4.55 g/kg SID

Ca concentrations, but these were superior to those fed the lower SID Ca concentrations.

Overall, these predicted values indicate that the growth of broiler growers is maximized below the current Ross (2019) Ca recommendation (8.7 g/kg total Ca).

Requirements for SID Ca to Maximize Standardized Ileal Digestibility and Intake of Ca and P

Adequate intake of Ca and P is essential for proper skeletal development. Dietary concentrations of both Ca and P greatly influence the digestibility and intake of these minerals (Rodehutscord et al., 2012; Abdollahi et al., 2016). In the current study, the digestibility and intake of SID Ca were influenced by SID Ca concentrations. Increasing dietary Ca concentration decreased the SIDC of Ca, but increased the SID Ca intake. The negative effect of increasing dietary Ca concentrations on Ca digestibility is attributed to excess Ca forming insoluble Ca-P complexes in the digestive tract (Selle et al., 2009). Walk et al. (2021b, 2022) also observed decreased standardized ileal Ca digestibility with increasing dietary SID Ca concentrations. In contrast, David et al. (2021c) found no effect of increasing dietary SID Ca concentration (3.3−5.5 g/kg) on the SIDC of Ca in broiler starters. Possible reasons for this discrepancy may include the use of the narrower range of dietary Ca concentration in the starter phase and the age effect.

In the current study, the digestibility and intake of SID P were influenced by the main effects of both the SID Ca and SID P. A positive correlation was observed between SID P intake and SIDC of P (r = 0.75; $P < 0.001$) where both parameters were increased with increasing SID P concentrations. These findings are in agreement with those of Rodehutscord et al. (2012) and David et al. (2021c).

The SID Ca 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 SID Ca intake was negatively correlated with SID P intake (r = −0.24; $P < 0.01$) and SIDC of P (r = −0.49; $P < 0.001$). The negative influence of increasing dietary Ca on ileal P digestibility has been reported previously (Mutucumarana et al. 2014a; Walk et al., 2021b, 2022). Similarly, a reduced P absorption with increasing dietary Ca has been reported in young turkeys using yttrium-91 as a reference substance (Hurwitz et al., 1978).

In the current study, the ratio of SID Ca intake to SID P intake was influenced by the interaction between SID Ca and SID P, highlighting the importance of formulating diets with proper Ca to P ratio. At all SID Ca concentrations, the ratio increased with decreasing dietary SID P and increasing SID Ca. Such trends are to be expected as the SID Ca intake increased while the SID P intake decreased with increasing dietary SID Ca.

Requirements for Digestible Ca to Maximize Bone Mineralization

An important criterion that determines the bone strength is bone mineralization. Bone mineralization is usually assessed based on bone concentration of ash, Ca, and P, because 99% of Ca and 80% of P are deposited in the bone (Veum, 2010). In the current study, tibia ash was positively correlated with tibia Ca (r = 0.85; $P < 0.001$) and tibia P (r = 0.73; $P < 0.001$). As expected, the lowest concentration of SID Ca (1.80 g/kg) reduced the concentration of tibia parameters. Similarly, as expected, the lowest concentration of SID P (3.5 g/kg) reduced the concentration of ash and P in the tibia. In the current study, tibia ash was increased as the concentrations of both SID Ca and SID P increased. Similarly, David et al. (2021c) reported an increased tibia ash at 5.0 g/kg SID P compared to that at 4.0 g/kg SID P. Although the bone ash was increased with increasing dietary SID P in the current study, the weight gain was similar across different SID P (3.5−5.5 g/kg) at a range of SID Ca 2.35 to 4.00 g/kg. Furthermore, no leg abnormalities were observed in any dietary treatment, suggesting that 3.5 g/kg SID P is sufficient for broiler growers at SID Ca concentrations between 2.35 and 4.00 g/kg.

According to the response surface model analysis, the concentration of SID Ca that maximized tibia ash at 3.5 g/kg SID P was 3.69 g/kg (equal to 7.28 g/kg total Ca), which corresponded to SID Ca to SID P ratio of 1.05. This estimate is lower than the Ross (2019) total Ca recommendation for growers (8.7 g/kg total Ca) and that reported for broiler starters for tibia ash (4.51 g/kg SID Ca; David et al., 2021c), demonstrating a reduction of 18% in SID Ca requirement for maximized tibia ash in growers compared to broiler starters. In the current study, concentration of SID Ca that maximized the tibia Ca concentration at 3.5 g/kg SID P was 4.06 g/kg, which corresponded to SID Ca to SID P ratio of 1.16. These estimates are well above the requirement of SID Ca and the ratio of SID Ca to SID P for maximized weight gain (3.05 g/kg and 0.87, respectively), demonstrating that the birds require Ca and P for bone tissue synthesis beyond the needs for other body tissues (Gautier et al., 2017). Similar findings were reported in broiler starters (David et al., 2021c) and in growing pigs (González-Vega et al., 2016; Lagos et al., 2019). Recently, Walk et al. (2022) reported a range of SID Ca of 3.94 to 5.15 g/kg to maximize the bone ash for broiler growers (11−24-day-old) using different nonlinear prediction models (quadratic polynomial model, straight-broken line, and quadratic-broken line regressions). The predicted estimates were higher than the current estimate (3.69 g/kg). Using one of the above-mentioned prediction models, Walk et al. (2021b) similarly reported a higher SID Ca requirement of 5.30 g/kg for bone ash of 0 to 10-day-old broilers compared to the value (4.51 g/kg SID Ca) reported by David et al. (2021c) for broiler starters. Among the possible reasons for the discrepancy between these two studies, several are worth considering. First, different prediction models were employed and likely to be the major contributor. Second, their assay diets contained enzymes (phytase, protease, and carbohydrase). These enzymes, especially the phytase, would have confounded the estimates. Third, their studies considered aP values, but not the SID P. In addition, the SID requirement of both Ca and P should be on digestible basis. Fourth, the SID Ca requirement was estimated at a single P concentration (4.8 g/kg aP) in their studies. However, it was found in our current and previous (David et al., 2021c) studies that the requirement of digestible Ca is variable depending on the dietary SID P concentration. For instance, based on current findings, that the SID Ca requirement would be higher if the dietary P is higher. Finally, different strains of broilers were utilized in these studies; Arbor Acres plus by Walk et al. (2021b, 2022) whereas Ross 308 in our studies. However, whether these contemporary strains use Ca with different efficiencies cannot be established with any clarity due to lack of published data.

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

The ATTRC of Ca was unaffected by dietary SID P concentrations (3.5−5.5 g/kg), supporting the possible use of 3.5 g/kg SID P for broiler growers. The negative effect of increasing dietary SID Ca concentrations on the ATTRC of Ca may be explained by the formation of insoluble Ca-phytate complex, which makes Ca unavailable for absorption. In general, regardless of dietary treatments, the percentage Ca retention (38−64%) compared closely to that of the percentage Ca absorption (43−66%). These findings demonstrate that almost all absorbed dietary Ca was retained in the body. The strong positive correlation ($r = 0.72$; $P < 0.001$) between the ATTRC and SIDC of Ca lends further support to this proposition.

In the current study, the retained Ca (g/bird) increased with increasing dietary SID Ca, which could be due to the increased SID Ca intake. This increased SID Ca intake, a function of feed intake and dietary SID Ca, was due to the increasing dietary SID Ca. Because the feed intake was similar across most of the dietary treatments in the current study. In the current study, retained Ca was positively correlated with tibia ash ($r = 0.55$; $P < 0.001$) and tibia Ca ($r = 0.51$; $P < 0.001$). Retained Ca (g/bird) was higher at 4.00 g/kg SID Ca and was reduced beyond 4.00 g/kg SID Ca, highlighting the negative effect of excess Ca on the retention of Ca (Gautier et al., 2017). However, the Ca retention at or below 2.35 g/kg SID Ca concentration was lower than that at 4.55 g/kg, further highlighting the inadequate supply of SID Ca when it is at or below 2.35 g/kg. Nevertheless, based on the response surface analysis, retained Ca was maximized at 4.39 g/kg SID Ca, regardless of the SID P concentration.

At 4.5 and 5.5 g/kg SID P, the percentage P retention (52 and 44%, respectively) was substantially lower than the percentage P absorption (70 and 75%, respectively), indicating urinary excretion of absorbed P. Excess P is known to be excreted through the kidney when the dietary P is above the physiological threshold for maximum retention (Leske and Coon, 2002). On the other hand, the percentage of both the absorption (66%) and retention (65%) were similar at 3.5 g/kg SID P and this retention was higher than those at other SID P concentrations, further confirming the appropriateness of using 3.5 g/kg dietary SID P for maximum P retention in broiler growers. The ATTR of P was negatively correlated ($r = -0.46$; $P < 0.001$) with SIDC of P in the current study. David et al. (2021c) reported a SID P concentration of 5.0 g/kg in broiler starters for maximum retention of P, suggesting a reduced P requirement with advancing broiler age. In the current study, the retained P (g/bird) was positively correlated with weight gain ($r = 0.51$; $P < 0.001$), 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).

The SID Ca requirements of 4.9 and 3.8 g/kg have been reported by Walk et al. (2021b, 2022) for maximized P retention of broiler starters and growers, respectively. However, in the current study, the SID Ca requirement prediction for P retention was not made due to the linear Ca effect. Nevertheless, the SID Ca requirement of 3.65 g/kg (at 5 g/kg SID P) has been recommended for broiler starters in the previous study (David et al. (2021c) which is lower than the value (4.9 g/kg) reported by Walk et al. (2021b) for 1 to 10-day-old broilers.

In the current study, the ratio of retained SID Ca and retained SID P was positively correlated ($r = 0.58$; $P < 0.001$) 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 ratios ranged from 0.72 to 1.23 depending on the dietary SID Ca concentration. As discussed above, the weight gain and tibia ash were optimized at SID Ca to SID P ratios of 0.87 and 1.05, respectively. In the current study, the ratio of retained SID Ca and retained SID P was positively correlated with tibia ash ($r = 0.39$; $P < 0.001$) and tibia Ca ($r = 0.35$; $P < 0.001$), demonstrating the importance of Ca and P ratio on the deposition of bone minerals.

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

The Ca concentration of carcasses of 11- and 24-day-old broilers was 16.0 and 8.8 to 23.4 g/kg, respectively. Clearly, the feeding of diets varying in Ca and P concentrations resulted in the wide range of carcass Ca concentrations in 24-day-old broilers. Carcass Ca concentrations of 18.9 and 16.9 g/kg in 12- and 22-day-old birds, respectively, were reported by Caldas et al. (2019). Carcass P concentrations of 11- and 24-day-old broilers in the current study were 13.1 and 9.5 to 15.6 g/kg, respectively. Similarly, Caldas et al. (2019) reported carcass P concentrations of 15.3 and 13.7 g/kg in 12- and 24-day-old broilers. Carcass Ca and P retention was found to be reflective of the total tract Ca and P retention values. The retained (g/bird) carcass Ca ($r = 0.63$; $P < 0.001$) and P ($r = 0.28$; $P < 0.01$) was positively correlated with the total tract Ca and P retention (g/bird), respectively. Interestingly, at 3.5 g/kg SID P, carcass Ca retention, carcass P retention and tibia ash were maximized at the SID Ca concentrations of 3.87, 3.64, and 3.69 g/kg, respectively, suggesting a close association between tibia mineralization and carcass mineral retention.

In conclusion, the present data demonstrated that growth performance, bone mineralization and Ca and P utilization of broiler growers were optimized at 3.5 g/kg SID P concentration. As mentioned above, differences between calculated and determined SID Ca values were minimal at 3.5 g/kg SID P when the SID Ca was between 2.35 and 3.45 g/kg. The estimate of 3.5 g/kg SID P is lower than the current Ross (2019) recommendation for aP (4.35 g/kg). Growth performance was similar within the SID Ca concentrations of 2.90 to 4.00 g/kg regardless of the SID P concentrations, and within the SID Ca to SID P ratios of 0.53 to 1.14. The SID Ca required for maximum weight gain and tibia ash was 3.05 and 3.69 g/kg, respectively, at 3.5 g/kg SID P, which corresponded to SID Ca to SID P ratios of 0.87 and 1.05, respectively. The current Ross (2019) Ca recommendation (8.7 g/kg total Ca) for broiler growers is higher than the current estimates for weight gain (3.05 g/kg SID Ca or 6.11 g/kg total Ca) and tibia ash (3.69 g/kg SID Ca or 7.28 g/kg total Ca). Maximum bone mineralization requires more Ca than maximum growth performance.

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.2022.102135.

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