The effect of reducing dietary calcium in prestarter diets (0–4 D) on growth performance of broiler chickens, tibia characteristics, and calcium and phosphorus concentration in blood

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ABSTRACT During the incubation period, the Ca-to-P weight (mg/mg) ratio in the yolk increases from 0.26 on day 0 to 0.92 on day 17.5 and to 2.9 at hatch. Moreover, the absolute Ca content in the yolk increases by 41%, whereas P content decreases by 87%, from day 0 to the day of hatching. Thus, at hatch and during the first days after hatching, there are high reserves of Ca relative to P in the residual egg yolk, risking hypercalcemia and hypophosphatemia. A growth performance study was conducted to explore the effects of reducing dietary Ca content in the prestarter phase (0–4 D) on BW and bone mineral deposition during the first days after hatch and at market weight (day 37). Four prestarter (0–4 D) diets were formulated to have 0.4, 0.6, 0.8, and 1.0% Ca content. After the prestarter phase, all birds were fed with the same commercial diets based on a 3-phase feeding program (starter, grower, and finisher). Growth performance (BW, ADG, ADFI, and feed conversion ratio [**FCR**]) was monitored throughout the study, and blood and tibia bone samples were collected on specific days. On day 4, BW and ADG decreased with dietary Ca contents higher than 0.6% ($P < 0.05$), but there were no differences in BW on day 14 onward ($P > 0.10$). For the overall study (0–37 D), there were no differences in ADG and ADFI, but the FCR decreased with lower Ca contents ($P < 0.05$). On day 4, there were no differences in blood plasma Ca concentration, but P concentration increased in the group treated with diet containing 0.4% Ca compared with the groups treated with diets containing 0.6 and 0.8% Ca ($P < 0.05$). Tibia ash content decreased in the group treated with diet containing 0.4% Ca ($P < 0.05$) compared with all other treatments at the end of the prestarter phase. Tibia ash content and tibia breaking strength, on day 37, were not different among the treatments ($P > 0.10$). In conclusion, during the prestarter phase, BW increased with dietary Ca contents lower than 0.6%, most likely improving Ca–P balance; bone mineral deposition was reduced in this period. On feeding with a diet containing higher Ca content, bone mineral content was rapidly recovered.

Key words: broiler, calcium, performance, phosphorus, prestarter

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INTRODUCTION

Current commercial feeding programs do not precisely align with optimal nutrient requirements; broiler chickens are fed below or above daily requirements at the beginning or at the end of the feeding phase, respectively (Gutierrez et al., 2008). The mismatch between the nutrient requirements for maximal growth and the nutrient content of the feeding program could be more impactful on early growth stages, more so when birds are fed with the starter diet for a long period. Moreover, compared with research in amino acid and energy nutrition, there is considerably less information about mineral nutrition, especially in the immediate days after hatching (de Jong et al., 2017).

During embryo development, P is mainly stored in the egg yolk (Yair and Uni, 2011), whereas Ca is stored in the egg yolk and in the eggshell (Richards, 1997; Yair and Uni, 2011). Recently, Hopcroft et al. (2019) reported that Ca-to-P concentration ratio in the yolk increased from 0.257 at day 0 to 0.924 at day 17.5. In the same study, the absolute Ca content in the yolk increased from 29 to 50 mg, whereas P content decreased from

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115 to 55 mg. At hatch, it has been reported that more than 90% of the initial P content in the yolk has been used (Yair and Uni, 2011; Uni et al., 2012). During the first 24 h after hatch of early-hatched chicks, Ca concentration in the residual yolk increased by 46%, while P concentration remained the same; however, total Ca content (mg) decreased by 26%, whereas P content decreased by 45% (Hopcroft et al., 2020), implying a selective utilization of P compared with Ca. Overall, there are higher Ca reserves (relative to the embryo Ca utilization) than P reserves for embryo development, and at hatch, there are limited P reserves in the residual yolk.

Considering the depleted P reserves in the residual egg yolk, the high Ca reserves, and the intrinsic interaction between these minerals (Li et al., 2017), nutritional strategies to improve the Ca–P balance during the first days after hatch should be considered. Current starter diets do not account for the additional supply of Ca from the residual yolk (Hopcroft et al., 2017) and may further offset the Ca–P imbalance (i.e., hypercalcemia and hypophosphatemia). Furthermore, absorption of P and Ca is tightly regulated by the active form of vitamin D_3 (Proszkowiec-Weglarz and Angel, 2013; Li et al., 2017). Therefore, the high Ca-to-P ratio after hatch, worsened by regular starter diets (1% Ca), may have a negative impact on dietary P absorption. Moreover, dietary Ca can decrease phytase activity and form indigestible complexes with phytic P (Selle et al., 2009) and free fatty acids (Tancharoenrat and Ravindran, 2014), further decreasing P availability and compromising energy availability for the growing bird.

The NRC (1994) requirements for Ca are based on bone mineral deposition, and these recommendations do not necessarily match those for maximal growth performance. Indeed, in 14- to 28-day-old broilers, Driver et al. (2005) concluded that the Ca requirement for maximal BW gain is lower than the requirement for maximal bone ash content. The Ca and P requirements for young birds, especially immediately after hatch, should be further investigated. The present study explored the effects of reducing the dietary Ca content during the first 4 D of rearing on growth performance and on bone mineral deposition of broiler chickens. We hypothesized that the current dietary Ca contents in starter diets may aggravate the Ca–P imbalance after hatch, hindering availability of P and, consequently, early growth.

MATERIALS AND METHODS

Animals and Housing

The present study was approved by the Trouw Nutrition Animal Care Committee and followed recommendations of the Castile-La Mancha Animal Welfare Department (Royal decree RD 53/2013), in compliance with the European Union guidelines for the care and use of animals in research (European Parliament, 2010). In total, 4,960 one-day-old, male Ross 308 chickens (SADA Inc., Alcala del Rio, Seville, Spain), vaccinated against Marek and Gumboro diseases, were randomly distributed into 2 identical environmentally controlled rooms (14 m × 20 m, each room) with 40 equal floor pens each (2.38 m × 1.68 m; 62 chicks per pen). During the study, the barn temperature was maintained at 32°C at day 0, 30°C at day 1, and 29°C from day 2 to 4 and gradually decreased to 21°C by day 22. The lighting schedule was 23 h of light (1 h of darkness) during the first 4 D, followed by 16 h of light and 8 h of darkness thereafter. All floor pens were equipped with 5 functional drinking nipples, and fresh wood shavings were used as bedding material. Floor pens were assigned to one of the 4 prestarter dietary treatments (20 pens/treatment), according to a randomized complete block design, with a total of 20 blocks per treatment (10 blocks per room).

Treatments and Feeding Program

The dietary treatments consisted of 4 prestarter diets (0–4 D) with different Ca contents (0.4, 0.6, 0.8, and 1.0%). The prestarter diets met or exceeded NRC (1994) nutrient specifications for energy, amino acids, minerals (except Ca), and vitamins. Digestible P during the prestarter phase was maintained at 0.46% for all treatments, and no phytase enzyme was added to the diets. Before diet formulation, all ingredients were analyzed for CP, Ca, and P according to AOAC (2012). To reduce error of multiple mixings, the final experimental prestarter diets were made by mixing the diets with the lowest and highest Ca levels in different proportions; diet with the lowest Ca content represented 100, 66.7, 33.3, 0% and diet with the highest Ca content represented 0, 33.3, 66.7, and 100% of the prestarter diets containing 0.4, 0.6, 0.8, and 1.0% Ca, respectively. The diet with 1.0% Ca aimed to represent the Ca content of commercial starter diets and was considered the control treatment in the present study. Birds were fed with the experimental prestarter diets from placement (day 0) until the morning of day 4. Thereafter, all birds were fed with the same diets according to a commercial 3-phase feeding program: starter (4–7 D; the same diet as the 1% Ca control), grower (7–29 D), and finisher (29–37 D) (Table 1).

During the first 5 D, the respective diets were placed on feeding plates. Tower feeders were placed, one per pen, on day 4 until the end of the study. On days 4 and 5, the floor plate and the tower feeder were both inside the pen to ease transition from one feeding system to the other. Diets in all feeding phases were fed ad libitum, and birds had free access to fresh water throughout the study. Live BW and feed intake per pen were monitored daily during the prestarter phase and on days 7, 14, 21, 29, and 37. Feed intake was calculated as the weight difference between offered feed and leftovers collected from the feeders. Feed conversion ratio (**FCR**) was calculated as the ratio between average daily feed intake (**ADFI**) and average daily gain (**ADG**). In each of the monitoring days, 2 groups of 3 trained people, blinded to the treatment allocation, were required to perform the tasks

Table 1. Ingredient composition and calculated nutrient content of the basal diets in the prestarter phase (0–4 D) and the starter (5–7 D), grower (7–29 D), and finisher diets (29–37 D).

Ingredient, %[1]	0.4% Ca[2]	1.0% Ca[2] (starter)	Grower	Finisher
Corn	57.10	57.10	30.00	17.50
Wheat			35.35	51.27
Soybean meal (48% CP)	31.46	31.46	26.70	23.44
Soybean oil	3.450	3.450	4.056	5.207
Corn gluten meal	3.400	3.400	0.960	0.061
Salt	0.092	0.092	0.177	0.183
Monocalcium phosphate	1.098	1.076	0.412	
Monosodium phosphate	0.744	0.762	0.354	0.300
Calcium carbonate		1.613	0.707	0.785
Sodium bicarbonate	0.011			0.024
L-Lysine HCl (98%)	0.205	0.205	0.217	0.209
DL-Methionine (99%)	0.280	0.280	0.278	0.267
L-Threonine (98%)	0.003	0.003	0.043	0.053
Vitamin and mineral premix[3]	0.500	0.500	0.500	0.500
Sepiolite	1.599			
Commercial phytase			0.100	0.100
Commercial NSPase			0.100	0.100
Coccidiostat[4]	0.063	0.063	0.050	
Total	100.00	100.00	100.00	100.00
Calculated nutrient and energy content of the diet, %[5]				
AME, kcal/kg	2,850	2,850	2,925	3,000
Moisture	11.6 (7.9)[5]	11.5 (7.9)	10.6 (8.1)	10.0
Ash	6.0 (6.0)	6.1 (5.8)	4.6 (4.5)	4.2
CP	21.4 (21.2)	21.4 (20.7)	19.8 (19.8)	18.7
EE	6.5 (5.5)	6.5 (5.9)	6.3	7.1
CF	2.5	2.5	2.7	2.7
Digestible Lys	1.15	1.15	1.06	0.98
Digestible Met	0.55	0.55	0.51	0.48
Digestible Met + Cys	0.81	0.81	0.76	0.72
Digestible Thr	0.69	0.69	0.65	0.61
Digestible Trp	0.22	0.22	0.21	0.20
Digestible Ile	0.79	0.79	0.72	0.66
Digestible Val	0.88	0.88	0.80	0.74
Digestible Arg	1.22	1.22	1.13	1.06
Digestible Gly + digestible Ser	1.66	1.66	1.59	1.51
Ca	0.400 (0.465)	1.000 (0.965)	0.600	0.550
P	0.716 (0.725)	0.716 (0.755)	0.507	0.412
Digestible P	0.460	0.460	0.370	0.290
Inositol P	0.242	0.242	0.239	0.233
Na	0.170	0.170	0.140	0.140
K	0.900	0.900	0.850	0.800
Cl	0.150	0.150	0.200	0.200

Abbreviations: CF, crude fiber; EE, ether extract.

[1]Units for all items unless specified.

[2]To obtain 0.4, 0.6, 0.8, and 1.0% Ca contents (prestarter experimental diets), diets with the lowest (0.4%) and highest (1.0%) Ca contents were mixed at 100, 66.7, 33.3, 0% and 0, 33.3, 66.7, and 100% of the final diets.

[3]Premix provides the following per kg of complete diet: 10,000 IU, vitamin A (trans-retinyl acetate); 2,500 IU, vitamin D3 (cholecalciferol); 50 IU, vitamin E (all-rac-tocopheryl-acetate); 2.0 mg, vitamin B1 (thiamine mononitrate); 6 mg, vitamin B2 (riboflavin); 40 mg, vitamin B3 (niacin); 4.0 mg, vitamin B6 (pyridoxine HCl); 25 mcg, vitamin B12 (cyanocobalamin); 2.0 mg, vitamin K3 (bisulfate menadione complex); 10 mg, pantothenic acid (d-Ca pantothenate); 1.0 mg, folic acid; 150 mcg, d-biotin; 0.25 mg, Se (Na_2SeO_3);1.0 mg, I; 15 mg, Cu ($CuSO_4 \cdot 5H_2O$); 67.7 mg, Fe ($FeSO_4 \cdot 7H_2O$); 90 mg, Mn ($MnSO_4 \cdot H_2O$); 80 mg, Zn (ZnO).

[4]Commercial coccidiostat provided 80 g of narasin and 80 g of nicarbazin per kg of the product.

[5]Analyzed values are presented within brackets below the corresponding calculated value. Calculated values for CP, Ca, and P are derived from analyzed values of individual ingredients, as presented in Supplemental Table 1.

within 1.5 h during the first week and 2.5 h for the rest of the study period.

Sampling and Calculations

Immediately after placement and before feeding, 2 random birds per pen were euthanized by cervical dislocation for blood sampling and tibia bone collection. On day 1, 4 random birds were euthanized per pen; blood was collected from 2 of these birds, and tibia bones were sampled from all 4 birds. On days 2, 3, 4, 7, 14, 21, and 37, 2 random birds per pen per day were euthanized, and tibia bones were collected from all birds; blood samples were also collected on days 2, 4, and 7 from these birds.

Overall, bird count per pen before each sampling (data used for growth performance calculations) was 62, 52, 50, 48, 46, and 44 on days 0, 4, 7, 14, 21, and 37, respectively.

Immediately after cervical dislocation, blood samples were collected by making a slit in the neck area and collected in clean tubes (3 to 5 mL, depending on the age of the bird). Blood tubes were centrifuged at 2,500 × *g* at 4°C for 10 min. Blood serum was harvested immediately and mixed in equal proportions per pen per day before freezing; serum samples were stored at −20°C until analyzed for Ca and total P concentrations (Analisis Clinicos Lab, Madrid, Spain).

The tibia bones were dissected and cautiously cleaned of any residual meat. Using the whole right tibia, dry matter and ash content (%) were determined. Ash content in the tibia (g) was calculated as the product of the dry matter weight (g) and the ash content in the tibia (%). The left tibia bones were used to determine breaking strength (model: TA-XT plus; Stable Micro Systems, Godalming, Surrey, United Kingdom). Tibia breaking strength was only determined on days 14, 21, and 37.

Statistical Analysis

Normality of residuals was determined using the UNIVARIATE procedure of SAS (version Studio, SAS Institute Inc., Cary, NC). Outliers were determined using the INFLUENCE statement of the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Block was considered a random effect, and the pen was considered the experimental unit for all variables. Whenever measurements were taken on multiple chickens per pen, the SUBJECT statement was used to indicate that the pen was the experimental unit. The main effect of the treatment was determined using the MIXED procedure of SAS, and differences between the least square means were determined using the SIMULATE statement in SAS. Significant differences were stated when $P \leq 0.05$ and a trend when $P \leq 0.10$.

RESULTS

Growth Performance During the Prestarter Phase

On arrival (day 0) and on day 1, there were no differences in BW among the experimental treatments ($P > 0.10$; Table 2). On day 2, BW of the control group (1.0% Ca) was 1.3 and 1.2% lower than that of the groups treated with diet containing 0.4 and 0.6% Ca, respectively ($P < 0.05$); the group treated with diet containing 0.8% Ca showed intermediate results, and the result was not different compared with that of the other treatment groups. On day 4 (end of the prestarter phase), BW was similar in the groups treated with diet containing 0.4 and 0.6% Ca ($P > 0.10$), but significantly decreased at higher Ca levels ($P < 0.05$).

ADG was not affected during the first day ($P > 0.10$). On day 2, ADG was the highest in the group treated with diets containing 0.4% Ca and the lowest in the control (1% Ca) treatment group. On days 3 and 4 and in the overall prestarter period (days 0–4), ADG was not different between the groups treated with diet containing 0.4 and 0.6% Ca ($P > 0.10$), and it was significantly lower in the control treatment group than in the groups treated with diet containing 0.4 and 0.6% Ca. ADG in the group treated with diet containing 0.8% Ca was intermediate and significantly higher ($P < 0.05$) than that in the control treatment group on days 3 and 4.

On day 1, ADFI increased in the group treated with diet containing 0.4% Ca compared with all other treatment groups ($P < 0.05$). There was a trend ($P = 0.055$) for higher ADFI in the group treated with diet containing 0.4% Ca than in the group treated with diet containing 1% Ca on day 3. For the overall prestarter period (day 0–4), ADFI was 3.2% higher in the group treated with diet containing the lowest Ca (0.4%) content than in the control group ($P = 0.009$); the other treatment groups showed intermediate results ($P > 0.10$).

Feed conversion ratio, on day 1, increased in the group treated with diet containing 0.4% Ca compared with other dietary treatment groups ($P < 0.05$). On day 2, the control treatment yielded the highest FCR compared with other treatments ($P < 0.05$); FCR in the groups treated with diet containing 0.4 and 0.6% Ca were the lowest. On day 3, FCR was not affected ($P = 0.248$), but on day 4, the groups treated with diet containing 0.4 and 0.6% Ca diets had similar FCR ($P > 0.10$) and showed lower FCR ($P < 0.05$) than the groups treated with diet containing 0.8 and 1.0% Ca. Overall (day 0–4), FCR decreased in the groups treated with diet containing 0.4 and 0.6% Ca compared with the control group ($P < 0.05$).

Growth Performance per Week

On days 7 and 14, average BW increased by 3.9 and 4.5% in the groups treated with diet containing 0.4 and 0.6% Ca compared with the control group, respectively ($P < 0.05$; Table 3). There were no differences ($P > 0.10$) in BW on any other day. During the first week (including the prestarter phase), ADG increased when chicks were fed with the prestarter diets containing 0.4 and 0.6% Ca compared with the control diet ($P < 0.05$); the group treated with diet containing 0.8% Ca showed intermediate results, and the result was different compared with that of all other treatment groups ($P < 0.05$). In the following weeks and the overall period (day 0–37), ADG was not different among treatment groups ($P > 0.10$). ADFI, during the first week (day 0–7), was higher in the groups treated with diet containing 0.4 and 0.6% Ca than in the control treatment group ($P < 0.05$). In the following weeks and the overall period (day 0 to 37), ADFI was not significantly different among the treatment groups ($P > 0.10$).

During the first week (day 0–7), FCR in the group treated with diet containing 0.6% Ca was lower than

Table 2. Body weight, ADG, ADFI, and FCR of chickens fed with different dietary Ca content in the prestarter diet (0–4 D).

BW, g	Dietary Ca, %					
Day	0.4	0.6	0.8	1.0, control	SEM	*P*-value
0	41.4	41.4	41.4	41.5	0.14	0.770
1	51.0	51.2	51.2	51.2	0.13	0.760
2	61.4[a]	61.3[a]	61.1[a,b]	60.6[b]	0.22	0.008
3	75.9[a]	76.0[a]	75.3[a]	74.2[b]	0.27	<0.001
4	93.6[a]	93.4[a]	91.9[b]	89.8[c]	0.36	<0.001
ADG, g						
Day	0.4	0.6	0.8	1.0	SEM	*P*-value
0–1	9.71	9.73	9.76	9.69	0.13	0.952
1–2	10.3[a]	9.96[b]	9.87[b]	9.40[c]	0.15	<0.001
2–3	14.4[a,b]	14.8[a]	14.2[b]	13.7[c]	0.18	<0.001
3–4	17.6[a]	17.4[a,b]	16.8[b]	15.8[c]	0.19	<0.001
ADFI, g						
Day	0.4	0.6	0.8	1.0	SEM	*P*-value
0–1	5.91[a]	5.38[b]	5.40[b]	5.26[b]	0.058	<0.001
1–2	9.36	9.05	9.27	9.22	0.10	0.158
2–3	15.6	15.5	15.4	14.8	0.23	0.055
3–4	21.6	21.2	21.7	21.3	0.29	0.372
FCR, g/g						
Day	0.4	0.6	0.8	1.0	SEM	*P*-value
0–1	0.609[a]	0.552[b]	0.555[b]	0.543[b]	0.007	<0.001
1–2	0.908[c]	0.913[b,c]	0.941[b]	0.984[a]	0.011	<0.001
2–3	1.088	1.05	1.085	1.086	0.015	0.248
3–4	1.211[b]	1.218[b]	1.298[a]	1.345[a]	0.019	<0.001

Overall (0–4 D)	0.4	0.6	0.8	1.0	SEM	*P*-value
$BW_{Initial}$, g	41.4	41.4	41.4	41.5	0.14	0.770
BW_{4d}, g	93.6[a]	93.4[a]	91.9[b]	89.8[c]	0.36	<0.001
ADG, g	13.0[a]	12.9[a]	12.6[a,b]	12.1[b]	0.08	<0.001
ADFI, g	12.9[a]	12.5[a,b]	12.7[a,b]	12.5[b]	0.10	0.009
FCR, g/g	0.990[b,c]	0.970[c]	1.007[a,b]	1.024[a]	0.009	<0.001

[a–c]Values in the same row without a common superscript letter significantly differ, $P \leq 0.05$.

Abbreviations: ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.

Values are least square means and SEM.

that in groups treated with diet containing 0.8 and 1.0% Ca ($P < 0.05$), and the group treated with diet containing 0.4% Ca showed intermediate results, and the result was not significantly different ($P > 0.10$) compared with that of all other treatment groups. During week 2, FCR was higher in the group treated with diet containing 0.4% Ca than in the control group ($P < 0.05$); the groups treated with diet containing 0.6 and 0.8% Ca showed intermediate results and, the results were not significantly different ($P > 0.10$). During weeks 3 to 5, FCR was not different ($P > 0.10$) among the treatment groups. For the overall period (day 0–37), FCR decreased in the groups treated with diet containing 0.4 and 0.8% Ca compared with the control treatment group ($P < 0.05$). Feed conversion ratio in the group treated with diet containing 0.6% Ca was not different compared with any other treatment groups ($P > 0.10$). Mortality in the whole study was not affected by the different Ca contents in the prestarter diets ($P > 0.10$).

Tibia Bone Characteristics and Ca and P Concentration in Blood Serum

Weight of tibia bones (absolute and relative to BW) was not affected by the different dietary Ca contents in the prestarter phase or on the following days ($P > 0.10$; Table 4). The mineral ash content in the tibia, measured on days 3 and 4, decreased in the group containing the lowest Ca content compared with all other treatment groups ($P < 0.05$; Figure 1). The effect of Ca content in the prestarter diets on tibia mineral ash content was not significant after the diet was replaced by the starter diet (1% Ca) ($P > 0.10$). The ash mineral content relative to dry weight of the tibia was low in the groups treated with diet containing 0.4 and 0.6% Ca compared with the groups treated with diet containing 0.8 and 1.0% Ca on days 2 and 3 ($P < 0.05$). On day 4, the relative ash content was the lowest in the group treated with diet containing 0.4% Ca compared with all other treatment groups ($P < 0.05$); the group treated with diet

Table 3. Body weight, ADG, ADFI, and FCR until the end of the study (day 37) of chickens fed with different dietary Ca contents in the prestarter diet (0–4 D).

BW, g	Prestarter dietary Ca, %					
Day	0.4	0.6	0.8	1.0, control	SEM	*P*-value
0	41.4	41.4	41.4	41.5	0.14	0.770
7	161[a]	162[a]	159[b]	155[c]	0.73	<0.001
14	478[a]	480[a]	477[a,b]	470[b]	2.0	0.002
21	1,003	1,005	1,004	996	4.1	0.300
29	1,836	1,843	1,847	1,834	8.8	0.462
37	2,601	2,616	2,617	2,615	11	0.649
ADG, g						
Day	0.4	0.6	0.8	1.0	SEM	*P*-value
0–7	17.2[a]	17.2[a]	16.7[b]	16.2[c]	0.10	<0.001
7–14	45.1	45.6	45.6	45.3	0.21	0.209
14–21	74.8	75.0	75.3	75.2	0.38	0.723
21–29	104.2	104.9	105.0	104.8	0.73	0.566
29–37	95.6	96.6	96.3	97.6	0.89	0.352
ADFI, g						
Day	0.4	0.6	0.8	1.0	SEM	*P*-value
0–7	17.2[a]	17.0[a,b]	16.8[b]	16.4[c]	0.10	<0.001
7–14	51.9	52.2	52.0	51.5	0.25	0.176
14–21	94.7	94.4	94.6	94.3	0.38	0.900
21–29	138	140	139	138	0.7	0.065
29–37	170	171	171	173	1.2	0.321
FCR, g/g						
Day	0.4	0.6	0.8	1.0	SEM	*P*-value
0–7	1.002[a,b]	0.991[b]	1.006[a]	1.015[a]	0.0058	<0.001
7–14	1.151[a]	1.145[a,b]	1.143[a,b]	1.138[b]	0.0027	0.005
14–21	1.263	1.260	1.256	1.254	0.0036	0.057
21–29	1.326	1.333	1.322	1.324	0.0051	0.162
29–37	1.781	1.768	1.775	1.773	0.0100	0.801

Overall (0–37 D)	0.4	0.6	0.8	1.0	SEM	*P*-value
$BW_{Initial}$, g	41.4	41.4	41.4	41.5	0.14	0.770
$BW_{37\ D}$, g	2,601	2,616	2,617	2,615	11	0.649
ADG, g	69.0	69.6	69.5	69.4	0.27	0.451
ADFI, g	96.7	97.7	97.1	97.9	0.39	0.122
FCR, g/g	1.401[b]	1.405[a,b]	1.400[b]	1.412[a]	0.0032	0.020
Mortality, %	2.96	2.62	1.82	3.62	0.602	0.219

[a-c]Values in the same row without a common superscript letter significantly differ, $P \leq 0.05$.
Abbreviations: ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.
Values are least square means and pooled SEM.

containing 0.6% Ca showed intermediate results, and the result was significantly different compared with that of the control group ($P < 0.05$). On day 7 (Figure 1) and on any following day (data not shown), the relative ash content did not change among treatments ($P > 0.10$). The strength needed to break the tibia bone, measured on days 14, 21, and 37, was unaffected by the different Ca contents during the prestarter phase ($P > 0.10$).

Blood serum Ca concentrations, measured on arrival (day 0) and on days 1, 4, and 7, were unaffected by the different dietary Ca contents in the prestarter diets ($P > 0.10$). Total P concentration in blood serum was not affected by dietary Ca content on day 1, but on day 4, birds fed with the diet containing 0.4% Ca had higher P concentration in blood serum than those fed with diets containing the 0.6 and 0.8% Ca ($P < 0.05$); the control group (1.0% Ca) was not different compared with other treatment groups ($P > 0.10$). There were no effects on blood serum P concentration on day 7 ($P > 0.10$).

DISCUSSION

The present study explored the effects of reducing dietary Ca content on growth performance of broiler chickens during the prestarter phase (0–4 D). The results of this study showed that reduction of dietary Ca content (digestible P = 4.6 g/kg) from placement to day 4 improved ADFI and ADG despite reduced bone mineral deposition. Mortality was low, and no signs of skeletal lameness or abnormalities were observed. The results indicate that the dietary requirements of Ca for bone mineral deposition and BW gain are not consistent, at least during the first 4 D. Studies on Ca and P requirements in very young chicks are not commonly reported, and the current dietary Ca

Table 4. Fresh weight, ash content, and breaking strength of tibia bones of broiler chickens fed with different dietary Ca contents during the prestarter phase (0–4 D)[1].

Tibia, g	Prestarter dietary Ca, %					
Day	0.4	0.6	0.8	1.0, control	SEM	*P*-value
Placement	0.278 ±	0.021				
1	0.319	0.313	0.314	0.319	0.0043	0.646
2	0.379	0.381	0.375	0.380	0.0054	0.856
3	0.486	0.492	0.489	0.488	0.0062	0.903
4	0.551	0.558	0.567	0.573	0.0094	0.378
7	1.13	1.12	1.10	1.10	0.017	0.428
14	2.36	2.38	2.39	2.43	0.042	0.668
21	5.12	5.04	5.15	5.02	0.089	0.609
37	12.4	13.1	12.9	12.8	0.23	0.298
Tibia, % BW						
Day	0.4	0.6	0.8	1.0	SEM	*P*-value
Placement	0.673 ±	0.054				
1	0.618	0.609	0.610	0.617	0.0084	0.815
2	0.617	0.621	0.607	0.622	0.0081	0.570
3	0.638	0.639	0.638	0.646	0.0084	0.875
4	0.599	0.613	0.613	0.619	0.0099	0.527
7	0.711	0.714	0.701	0.721	0.0096	0.517
14	0.467	0.470	0.472	0.476	0.0058	0.757
21	0.494	0.483	0.495	0.487	0.0047	0.233
37	0.479	0.490	0.490	0.489	0.0049	0.351
Tibia ash, mg						
Day	0.4	0.6	0.8	1.0	SEM	*P*-value
Placement	26.2 ±	0.80				
1	28.3	27.8	28.6	28.4	0.27	0.150
2	36.9	37.1	38.2	37.6	0.53	0.206
3	46.4[b]	48.6[a]	49.8[a]	49.9[a]	0.61	<0.001
4	56.8[b]	61.2[a]	62.8[a]	63.8[a]	0.86	<0.001
7	131	133	131	128	1.9	0.266
14	427	427	431	435	7.9	0.867
21	1,004	997	1,001	982	19	0.760
37	2,495	2,579	2,582	2,568	46	0.504
Breaking strength,[2] kg						
Day	0.4	0.6	0.8	1.0	SEM	*P*-value
14	7.6	7.3	7.5	7.5	0.19	0.672
21	19.6	18.4	19.1	19.0	0.53	0.349
37	28.5	28.0	26.6	28.5	0.94	0.386

[a,b,c]Values in the same row without a common superscript letter significantly differ, $P \leq 0.05$.

[1]Values are least square means and pooled SEM. Placement data represent average ± SD.

[2]Breaking strength of tibia was not determined prior day 14.

requirements (NRC, 1994) are based on maximal bone mineralization of broilers during the growing phase. Driver et al. (2005) reported, in a dose–response study, that the Ca requirement for maximal ADG in broilers, measured from day 0 to 16, was lower than 0.625% and lower than the requirement for maximal bone mineral deposition (1.00%; NRC, 1994). Similarly, Hamdi et al. (2015) concluded that from day 7 to 14, chickens had higher ADG when fed on a diet containing 0.7% Ca than when fed on a diet containing 0.9% Ca. Therefore, it could be implied that formulating diets with Ca content to achieve maximal bone mineralization may hinder BW gain (Li et al., 2017). It should also be noted that in the aforementioned studies and in other studies (Fallah et al., 2018), feeding chickens with low Ca levels for longer periods (more than 2 wk in those studies) could lead to a decline in bone mineralization. In the present study, although, on day 4, there was a decrease in tibia ash content in the group treated with diet containing the lowest Ca content (0.4% Ca), such difference was no longer observed after the birds were fed on a diet containing higher Ca content (1%) for only 3 D. To maximize BW at day 4, inclusion of 0.6% Ca in the diet appears to be optimal as 0.6% Ca content yielded similar BW at day 4, and it did not affect tibia ash mineral content when compared with the treatment with diet containing 0.4% Ca.

The negative effects of high dietary Ca content on decreasing BW gain have not been completely elucidated. However, multiple studies have concluded that when diets have a high Ca content, Ca in the digesta can reduce dietary nutrient availability and energy retention by multiple mechanisms (Lonnerdal et al., 1989; Selle et al., 2009; Walk et al., 2012; Paiva et al., 2013). Moreover, starter diets are not specifically formulated for newly hatched chicks because requirements have been established considering the first 2 wk of rearing. After hatch, the chick depends on dietary nutrients for growth, but during the first days after hatch, there is an extra supply of Ca and very little supply of P from the residual yolk (Yair and Uni, 2011; Hopcroft et al. 2020). Hopcroft et al. (2020) reported that at hatch, the Ca-to-P weight ratio in the yolk is as high as 2.9. From the residual egg yolk, the chick is able to use 52% of Ca, but more than 87% of P in the first 3 D, implying a selective and higher utilization of P than Ca. A diet formulated specifically for the prestarter phase could improve the imbalanced Ca–P status of newly hatched chicks.

The experimental diets did not include phytase and were formulated on a digestible P basis (0.46%). Thus, the negative interaction between the dietary Ca and phytase activity was avoided (Sommerfeld et al., 2018), and available P supply could be better predicted across dietary Ca contents. Assuming a Ca and P utilization rate of 7.37 and 4.37 mg/day from the residual yolk during the first 3 D (Hopcroft et al., 2017), the prestarter diets in the present study temporarily decreased the Ca-to-P ratio to as low as 1:1 in the group treated with diet containing 0.4% Ca (compared with 2.1:1 in the control group). Moreover, if it is considered that during the first days after hatch, the visceral organs develop intensively (Sklan, 2001; Lilburn and Loeffler, 2015), the necessity of Ca may be lower than the necessity of P, and the Ca-to-P concentration ratio of 2:1 (optimal for mineral deposition; Mello et al., 2012) may not apply in the very early stages of growth. Overall, feeding unnecessarily high Ca in prestarter diets may worsen Ca–P status, impeding P utilization and hindering growth performance. Nutritional strategies that can provide more bioavailable P (e.g., reduction of dietary Ca, suprasupplementation of phytase, reduction of Ca-to-P ratio; Driver et al., 2005; Tamin et al., 2004; Shafey, 1993) may have positive effects.

We did not find significant differences in serum Ca concentration with varying levels of dietary Ca, but the increased serum P concentration in the group treated

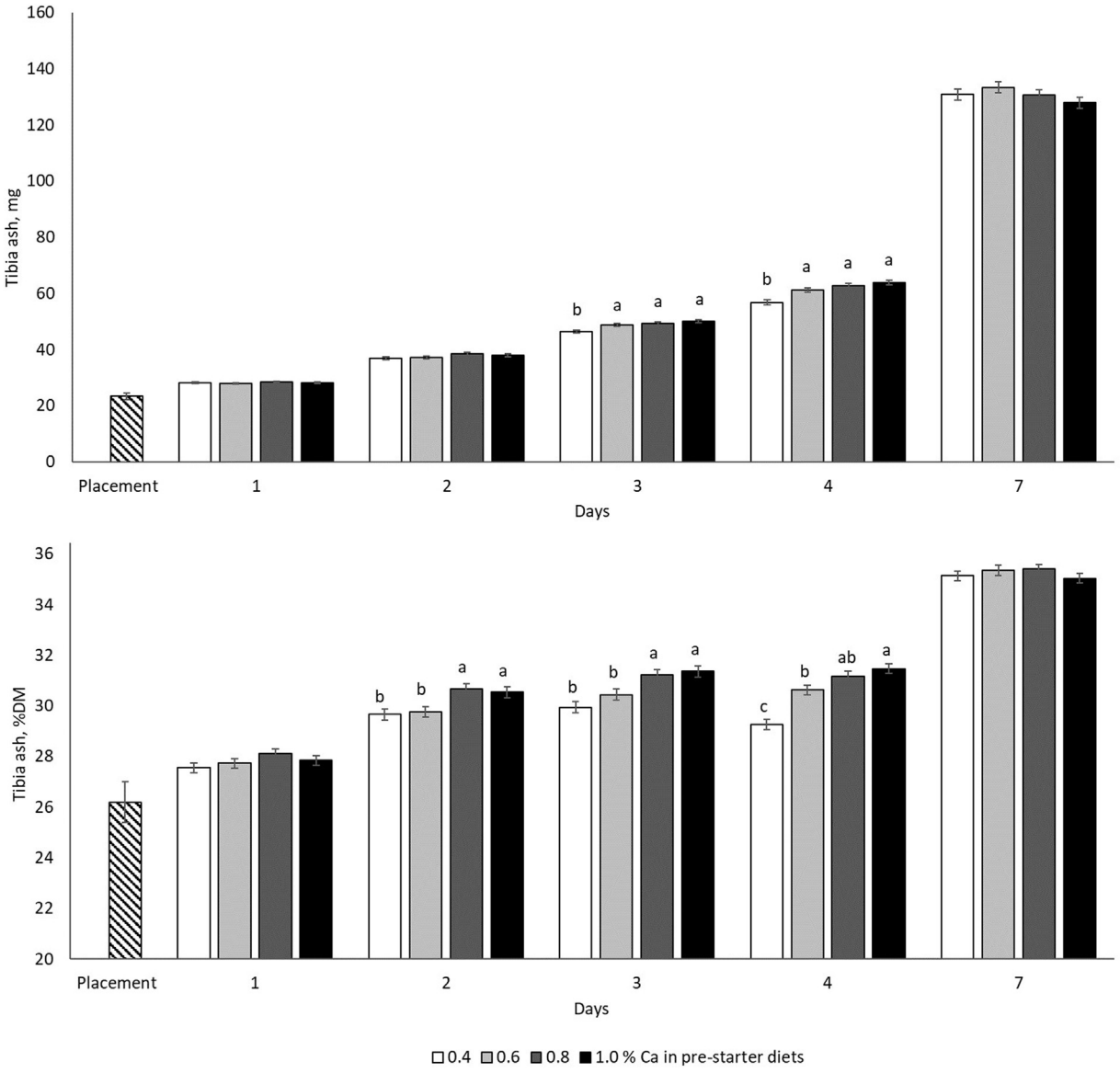

Figure 1. Tibia ash content during the first week of chickens fed with diets with different Ca levels during the prestarter phase (day 0–4). Values are least square means ± SE; placement data represent average ± SD. [a,b]Different superscripts within days differ significantly, $P < 0.05$.

with diet containing the lowest level of Ca (0.4%) is in agreement with results reported by Fallah et al. (2018). The low dietary Ca-to-P ratio may have increased P absorption in response to the increased secretion of vitamin D to increase active transport of luminal Ca (Lips, 2012). It is also possible that the higher concentration of serum P, seen in the group treated with diet containing the lowest Ca content, is related to lower bone formation, as indicated by the lower tibia ash content. In the body, 80% of P is stored in the form of hydroxyapatite in bones, whereas Ca in bones represents >99% of the total body content (Veum, 2010). Therefore, the higher bone mineralization when feeding high-Ca diets in the prestarter phase may be interpreted as a mechanism to buffer the excess of absorbed Ca and to maintain normal Ca levels in blood, but with the consequential reduction of P availability to other tissues. The complex hormonal mechanisms of Ca homeostasis (reviewed in detail by Proszkowiec-Weglarz and Angel, 2013 and Mundy and Guise, 1999) allow the chicken to increase dietary Ca utilization (i.e., increase absorption and reduce excretion; Paiva et al., 2013; Yan et al., 2005; Centeno et al., 2004) and maintain growth on a range of dietary Ca supply, including short deprivation of Ca (Yan et al., 2005). From the present study, this adaptation to low dietary Ca content appears to be activated at a very young age as there were no differences in bone ash content on day 7. It has also been reported that the adaptation capacity to increase utilization of Ca in chickens fed Ca-deficient diets appears to linger after the restriction period (Bar et al., 2003). An optimum dietary Ca reduction and length of the reduction warrants further investigation to improve dietary P and Ca utilization and growth performance without compromising the development of the bone structure.

It has been suggested that under different levels of Ca and P, broiler chickens tend to maintain Ca intake over P intake (Bradbury et al., 2014). The latter implies an

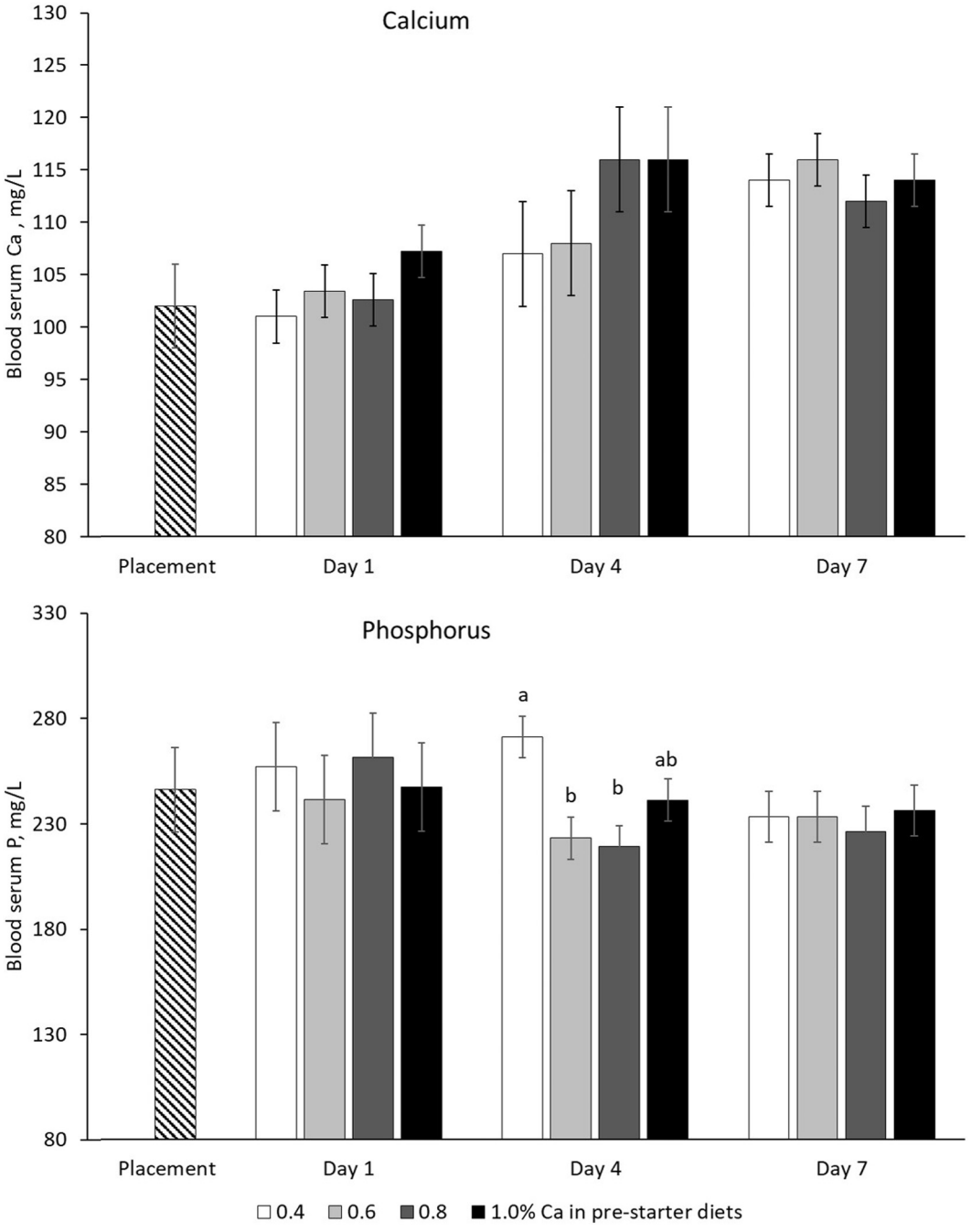

Figure 2. Blood serum Ca and P concentrations during week 1 of chickens fed with diets with different Ca levels during the prestarter phase (day 0–4). Values are least square means ± SE; placement data represent average ± SD. [a,b]Different superscripts within days differ significantly, $P < 0.05$.

increase in feed intake when there is a moderate restriction in dietary Ca content (from 1.2–0.64% Ca). This is in agreement with the ADFI reported during the first 4 and 7 D (Tables 2 and 3). This higher feed intake during the prestarter phase in the group treated with diet containing 0.4% Ca compared with the control group is also responsible for the increment in BW observed herein. The increased FCR observed on day 1 in the group treated with diet containing 0.4% Ca is underpinned by the higher feed intake on that day; during the following days, FCR consistently improved with lower Ca content during the prestarter phase as a result of higher ADG. In the present study, although there was a lack of treatment effect on final BW, FCR improved for the overall study as a result of lower FCR during weeks 1 to 3 (tendency to improve FCR on week 3; $P < 0.10$). Thus, specifically formulated diet for a prestarter phase can improve precision feeding in poultry, with beneficial impact on poultry producers.

In conclusion, the present study suggests that reducing Ca content in prestarter diets (0–4 D) can increase BW at week 1. The low bone mineralization observed in chicks fed on diet containing the lowest Ca content (0.4%) is rapidly compensated within the first days after being fed with a regular (1.0% Ca) diet, without further effects on tibia ash content and breaking strength. The improvements in BW during the prestarter phase (0–4 D) were not detectable after day 14, but

FCR decreased for the overall study (0–37 D). Thus, the improved robustness of chicks fed on low-Ca diets during the prestarter phase (0–4 D) did have commercial benefits for broiler chickens and could potentially be further improved by formulating specific prestarter diets. In the present study, treatment with 0.6% Ca in the prestarter diet improved BW and FCR similar to the treatment with 0.4% Ca, without affecting tibia ash mineral content at 4 D of age.

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Conflict of Interest Statement: WDM, RF-R, CAT, and AIG-R work(ed) at Trouw Nutrition during execution of the study; AD works at ForFarmers.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.psj.2020.05.056

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