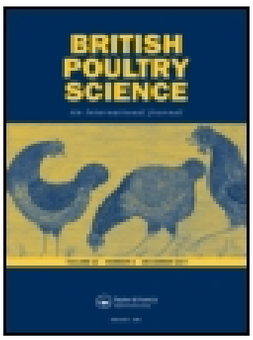

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Effects of dietary calcium and phosphorus restrictions on growth performance, intestinal morphology, nutrient retention, and tibia characteristics in broiler chickens

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ABSTRACT

1. This study evaluated the effects of dietary calcium (Ca) and available phosphorus (aP) restrictions on growth performance, intestinal morphology, nutrient apparent total tract retention (ATTR), and tibia characteristics.
2. A total of 1296, one-day-old male Ross-308 broilers were reared for 42 d. During the starter phase (1-10 d), all birds were fed a nutrient-adequate diet (C). Diets fed during the grower phase (11-24 d) included: 1. C; 2. 15% of the Ca and aP in C; 3. 30% of the Ca and aP in C. At the beginning of the finisher phase (25 d), chickens fed the C diet were divided into two subgroups including C, and C+ phytase (500 FTU/kg). Restricted treatments were divided into eight subgroups as 1. C; 2. 10% of the Ca and aP in C; 3. 20% of the Ca and aP in C; 4. 30% of the Ca and aP in C; 5. C+ phytase; 6. 10% of the Ca and aP in C+ phytase; 7. 20% of the Ca and aP in C+ phytase and 8. 30% of the Ca and aP in C+ phytase.
3. On d 24 and 42, ATTR of Ca and phytate phosphorus (pP) were linearly increased by decreasing Ca and aP levels ($P < 0.05$). Birds receiving phytase showed higher nutrient ATTR compared to those fed non-phytase supplemented diets ($P < 0.05$). Tibia Ca and P were linearly decreased at 24 d ($P < 0.05$) and tibial ash was linearly decreased ($P < 0.05$) at 42 d by decreasing levels of Ca and aP in finisher diets (without phytase). By decreasing the levels of Ca and aP in the finisher diets (with phytase) with a 30% reduction of Ca and aP in the grower phase, tibia ash linearly decreased ($P < 0.05$). Using 500 FTU/kg phytase improved tibia traits compared to non-phytase supplemented treatments ($P < 0.05$).
4. In general, decreasing dietary Ca and aP (up to 30%) during grower and finisher phases increased ATTR of minerals and decreased Ca, P and breaking strength (BS) of tibia without any negative effect on growth performance or intestinal morphology. Reduced dietary Ca and aP decreased tibial ash content, although 500 FTU/kg phytase improved ATTR of minerals and tibia attributes.

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Digestibility; gastrointestinal tract; ash; nutrition; bone mineralisation

Introduction

Phosphorus (P) is an essential mineral for skeletal integrity and is one of the most expensive components of poultry diets, after protein and energy, as its resources are limited in the world (Potchanakorn and Potter 1987). Due to the presence of P in cereals and oilseeds in the form of phytate and limited secretion of endogenous phytase in the digestive tract of poultry, this mineral is largely unavailable to birds (Viveros et al. 2002). In recent years, reducing feed costs while maintaining the performance of broilers with minimal environmental pollution has become a significant challenge for poultry nutritionists. In this regard, P is one of the problematic nutrients (Faridi, Gitoee, and France 2015). Researchers have concluded that enhancing P utilisation strategies, such as phytase addition and the use of low-phytate grains in the diet, has reduced the amount of P by 29 to 45% in excreta and litter (Angel et al. 2006). Simpson and Wise (1990) stated that the molar ratio of Ca to phytate in the diet is the main factor determining the intensity of phytate hydrolysis in the intestine due to its relationship to the formation of insoluble phytate-Ca complexes. It has been shown that high dietary Ca inhibits the hydrolysis of phytate in the intestine and decreases the availability of P by increasing the insoluble Ca-P complexes in digesta and excreta (Driver et al. 2005).

One of the nutritional strategies for effective use of P is to use the initial dietary restriction to increase the efficiency of using P in the diet (Letourneau-Montminy et al. 2008). The mechanisms involved in long-term adaptation of broilers to reduced dietary P and Ca are little known. According to previous studies, the expression of some genes encoding Ca and P transporters in intestinal enterocytes is influenced by long-term imbalances in these minerals (Ashwell and Angel 2010). It has been shown that high Ca levels exacerbates P deficiency and leads to loss of appetite, reducing the growth of soft tissue and bone (Driver et al. 2005). Research has shown that a mild reduction in dietary Ca to about 0.83% compared to 0.73% during 14 to 21 and 21 to 39 d of age has no detrimental effect on the performance of broilers (Ziaei et al. 2008). Imari et al. (2020b) studied broiler adaptation to low Ca and aP intake during the starter phase and found that nutrient ATTR was increased, but bone strength and mineralisation were impaired, although there was no change in growth performance.

Microbial phytase is commonly used in poultry diets to combat the anti-nutritional effects of phytate, improve the

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Table 1. Ingredients and nutrient composition of the experimental diets (as-fed basis).

	Starter (1-10 d)	Grower (11-24 d)			Finisher (25-42 d)			
		Reduction in calcium and available phosphorus levels (%)						
Ingredients (g/kg)	Control	0	15	30	0	10	20	30
Yellow maize	492.0	525.6	525.6	525.6	577.9	577.9	577.9	577.9
Soybean meal (44% protein)	415.6	377.8	377.8	377.8	322.9	322.9	322.9	322.9
Soybean oil	45.3	54.1	54.1	54.1	59.7	59.7	59.7	59.7
Dicalcium phosphate	19.3	17.1	13.3	9.4	15.4	13.1	10.7	8.4
Limestone	10.6	9.8	8.6	7.4	9.1	8.4	7.6	6.9
Vitamin premix[1]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Mineral premix[2]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Sodium bicarbonate	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Common salt	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9
L-Lysine HCl	2.5	1.7	1.7	1.7	1.8	1.8	1.8	1.8
DL-Methionine	3.8	3.2	3.2	3.2	2.9	2.9	2.9	2.9
L-Threonine	1.1	0.8	0.8	0.8	0.6	0.6	0.6	0.6
Choline chloride 60%	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Sand	-	-	5.0	10.1	-	3.0	6.1	9.1
Calculated nutrient composition (g/kg)								
Metabolisable energy (MJ/kg)	12.55	12.97	12.97	12.97	13.39	13.39	13.39	13.39
Crude protein	230.0	215.0	215.0	215.0	195.0	195.0	195.0	195.0
Lysine	14.4	12.9	12.9	12.9	11.6	11.6	11.6	11.6
Methionine	7.2	6.5	6.5	6.5	5.9	5.9	5.9	5.9
Methionine + cystine	10.8	9.9	9.9	9.9	9.1	9.1	9.1	9.1
Threonine	9.7	8.8	8.8	8.8	7.8	7.8	7.8	7.8
Calcium	9.6	8.7	7.4	6.1	7.9	7.1	6.3	5.5
Total phosphorus	7.4	6.8	6.2	5.5	6.3	5.9	5.5	5.1
Available phosphorus	4.8	4.4	3.7	3.0	4.0	3.6	3.2	2.8
Sodium	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Potassium	9.8	9.2	9.2	9.2	8.2	8.2	8.2	8.2
Chlorine	2.7	2.6	2.6	2.6	2.6	2.6	2.6	2.6
Dietary cation-anion difference (mEq/kg)	243.9	231.3	231.1	230.0	207.2	207.1	207.0	206.9

[1]Provided the followings per kg of diet: vitamin A (trans-retinyl acetate), 12500 IU; vitamin D_3 (cholecalciferol), 5000 IU; vitamin E (D L-α tocopherol acetate), 80 IU; vitamin K (menadione), 3.20 mg; riboflavin, 8.6 mg; pantothenic acid (D-Ca pantothenate), 18.6 mg; pyridoxine (pyridoxine-HCl), 4.86 mg; thiamine, 3.2 mg; vitamin B_{12} (cyanocobalamin), 0.02 mg; biotin, 0.25 mg; folic acid, 2.2 mg; nicotinic acid, 62.51 mg; ethoxyquin (antioxidant), 2.5 mg. [2]Provided the following per kg of diet: Fe, 20.23 mg; Zn, 110 mg; Mn, 120 mg; Cu, 16 mg; I, 1.25 mg; Se, 0.30 mg.

availability of P in the diet and reduce excretion *via* excreta. However, significant variation in the response to phytase has been observed due to factors such as substrate concentration, phytase level, intrinsic properties, phytase source, and feed particle size (Ravindran et al. 2006). Mondal, Panda, and Biswas (2007) showed that supplementation with phytase in a low P diet (0.3% *vs.* 0.45%) improved body weight, feed intake, feed conversion ratio and increased plasma P and Ca levels and tibia ash. However, further studies are needed to adjust the dietary Ca and P levels in the early stages of growth and fine-tune the appropriate phase for restriction.

Therefore, this study was performed to evaluate broiler chickens' responses to different dietary Ca and aP restriction regimens, with a constant, Ca: aP ratio of 2:1, during the grower and finisher phases. By decreasing Ca and aP in the grower diets, the treatments were expected to stimulate the adaptation mechanism of broilers to distinguish how much reduction could induce a more adaptive response. With different Ca and aP levels in the finisher phase, the study aimed to establish the possible reduction level of dietary P in response to Ca and aP restrictions of the grower phase. In addition, it was hypothesised that the birds would not be able to effectively adapt to reduced dietary Ca and aP levels, so a phytase was added to feed during the finisher phase to moderate any adverse effects of excessive reduction of Ca and P.

Table 2. Experimental design of dietary treatments during the grower and finisher phases.

Reduction in calcium and available phosphorus levels (%)	
Grower (11 to 24 d)	Finisher (25 to 42 d)
C[1]	C
	C + phytase[2]
15%C	C
	10%*C
	20%C
	30%C
	C + phytase
	10%C + phytase
	20%C + phytase
	30%C + phytase
30%C	C
	10%C
	20%C
	30%C
	C + phytase
	10%C + phytase
	20%C + phytase
	30%C + phytase

[1]Control diets contained the recommended levels of calcium and available phosphorus based on Ross 308 recommendations (Aviagen 2014b). [2]Phytase (Hostazym® P 5000) was used at a dose of 500 FTU/kg in experimental diets. *Digits show the dietary calcium and phosphorus reduction (%) based on Ross 308 recommendations (Aviagen 2014b).

Materials and methods

Ethical approval

All procedures were approved by the Animal Care and Use Committee of the Ferdowsi University of Mashhad, Mashhad, Iran.

Table 3. Calculated and determined total phosphorus (tP), available phosphorus (aP)[1], and calcium (Ca).

	tP		aP		Ca	
Diets	Calculated	Determined	Calculated	Determined	Calculated	Determined
Starter Control	0.74	0.73 ± 0.02	0.48	0.47 ± 0.02	0.96	0.96 ± 0.03
Grower Control	0.68	0.67 ± 0.01	0.44	0.42 ± 0.01	0.87	0.86 ± 0.01
Grower (15%[2] Control)	0.62	0.62 ± 0.02	0.37	0.36 ± 0.02	0.74	0.75 ± 0.01
Grower (30% Control)	0.55	0.56 ± 0.01	0.30	0.30 ± 0.01	0.60	0.59 ± 0.02
Finisher Control	0.63	0.63 ± 0.02	0.40	0.39 ± 0.02	0.79	0.79 ± 0.01
Finisher (10% Control)	0.59	0.58 ± 0.02	0.36	0.34 ± 0.02	0.71	0.73 ± 0.02
Finisher (20% Control)	0.55	0.55 ± 0.01	0.32	0.30 ± 0.01	0.63	0.64 ± 0.02
Finisher (30% Control)	0.51	0.51 ± 0.01	0.28	0.26 ± 0.01	0.55	0.56 ± 0.01

[1]The aP values were determined by subtracting analysed phytate P from analysed P. [2]Digits show the dietary Ca and P reduction (%) based on Ross 308 recommendations (Aviagen 2014b).

Table 4. Calculated and determined[1] phytase activity (FTU[2]/kg) recovered in finisher diets (25–42 d).

Treatments	Calculated	Determined
C[3]	0	62
C + phytase[4]	500	411
10%C[5] + phytase	500	452
20%C + phytase	500	486
30%C + phytase	500	433

[1]According to the method described by Yin, Zheng, and Kang (2007). [2]One FTU is defined as the amount of enzyme required to release 1 µmol of inorganic P per minute from sodium phytate at 37°C and pH 5.5. [3]Control diets contained the recommended levels of calcium and available phosphorus based on Ross 308 recommendations (Aviagen 2014b). [4]Phytase (Hostazym® P 5000). [5]The values of 10, 20, and 30% indicate the reduction of Ca and aP levels in experimental diets compared to control diet.

Table 5. Effects of reduction in dietary calcium (Ca) and available phosphorus (aP) levels (%) on the average body weight (AWB), daily weight gain (DWG), daily feed intake (DFI), feed conversion ratio (FCR), and jejunal morphology of broilers during the grower phase (11 to 24 d).

	Ca and aP reduction in grower diets				*P*-value		
	C[1]	15%	30%	SEM	Overall	Linear	Quadratic
ABW, 24 d (g/bird)	843.4	847.0	844.9	6.789	0.948	0.906	0.746
DWG (g/bird/day)	44.62	44.78	44.64	0.451	0.964	0.982	0.802
DFI (g/bird/day)	67.44	68.27	67.68	0.574	0.614	0.828	0.338
FCR (g:g)	1.52	1.53	1.52	0.008	0.700	0.866	0.416
Villus height (µm)	1122.8	1287.0	1266.0	73.84	0.262	0.190	0.322
Villus width (µm)	112.7	116.5	114.8	3.204	0.703	0.639	0.494
Crypt depth (µm)	185.7	201.5	207.5	11.36	0.396	0.194	0.728
V:C[2]	6.09	6.37	6.13	0.218	0.615	0.898	0.336
Muscle thickness (µm)	140.3	149.7	150.8	3.589	0.109	0.056	0.367
Villus surface area[3] (mm^2)	0.40	0.47	0.45	0.022	0.095	0.105	0.132

[1]Control diets contained the recommended levels of calcium and available phosphorus based on Ross 308 recommendations (Aviagen 2014b).
[2]V: C= villus height to crypt depth ratio. [3]Villus surface area was calculated by (2π) * (villus width/2) * (Villus height).

Birds, diets, and housing

A total of 1296, one-day-old male Ross 308 broiler chicks were obtained from a local commercial hatchery and reared for 42 d. The nutrient composition of the diets was extracted from the tables of the National Research Council (National Research Council 1994). Ingredients and nutrient composition are presented in Table 1. All birds were fed the same standard diet according to the Ross 308 nutrition guidelines up to 10 d (Aviagen 2014b). Then, the experimental diets were offered during 11–42 d (grower and finisher phases). Three experimental diets were fed to the birds during the grower phase including 1) control diet (C) with 12 replications of 12 birds each and, 2) diet with 15% restriction in Ca and aP compared to C (15%C) with 48 replications of 12 birds each and 3) diets with 30% restriction in Ca and aP compared to C (30%C) with 48 replications of 12 birds each.

At the start of the finisher phase (25 d), chickens fed the C diet were divided into two subgroups including 1) C, and 2) C + 500 FTU phytase per kg diet (Hostazym® P 5000, Huvepharma NV, Belgium). Where birds were fed the restricted diets during the grower phase, each was divided into eight subgroups during the finisher phase, *namely,* 1) C, 2) 10%C, 3) 20% C, 4) 30%C, 5) C + phytase, 6) 10%C + phytase, 7) 20%C + phytase, and 8) 30%C + phytase. The values of 10, 20, and 30% indicate the reduction of Ca and aP levels in experimental diets compared to the C diet (Table 2).

Table 6. Effects of dietary calcium (Ca) and available phosphorus (aP) levels (%) on average body weight (AWB), daily weight gain (DWG), daily feed intake (DFI), and feed conversion ratio (FCR) of broiler chickens during the finisher (25–42 d) and whole experimental period (11–42 d).

Experimental treatments (Dietary Ca and aP reduction)		Finisher (25-42 d)				Total experiment (11-42 d)		
Grower phase	Finisher phase	ABW (g/bird)	DWG (g/bird/day)	DFI (g/bird/day)	FCR (g:g)	DWG (g/bird/day)	DFI (g/bird/day)	FCR (g:g)
C[1]	C	2340.8	84.17	131.98	1.58	66.86	103.85	1.55
	C + phytase[2]	2355.9	85.20	133.63	1.59	67.45	104.56	1.55
15%	C	2344.7	84.94	133.10	1.59	66.53	103.98	1.56
	10%	2319.4	83.02	132.36	1.60	65.53	103.85	1.58
	20%	2339.9	83.74	134.60	1.61	66.36	104.77	1.58
	30%	2400.5	82.82	133.09	1.61	67.78	106.58	1.57
	C + phytase	2362.7	83.89	134.53	1.61	67.07	105.45	1.57
	10% + phytase	2360.1	84.99	134.53	1.59	66.96	105.21	1.57
	20% + phytase	2337.1	82.52	132.36	1.60	66.08	104.70	1.58
	30% + phytase	2339.3	81.98	132.30	1.62	66.12	104.52	1.58
30%	C	2371.0	84.14	136.22	1.62	66.96	106.50	1.59
	10%	2345.5	83.24	133.26	1.60	66.48	104.14	1.57
	20%	2316.9	81.90	132.75	1.62	65.29	104.01	1.59
	30%	2327.2	81.95	132.60	1.63	65.64	104.27	1.59
	C + phytase	2368.37	83.12	132.48	1.60	66.62	105.43	1.58
	10% + phytase	2349.5	82.82	133.17	1.61	66.42	104.52	1.57
	20% + phytase	2313.3	82.64	133.31	1.62	65.57	103.90	1.58
	30% + phytase	2336.7	83.05	130.97	1.59	66.14	103.03	1.56
SEM		23.737	0.931	1.748	0.013	0.697	1.282	0.012
P-Value		0.657	0.249	0.959	0.420	0.520	0.938	0.370

[1]Control diets contained the recommended levels of calcium and available phosphorus based on Ross 308 recommendations (Aviagen 2014b). [2]Phytase was used by 500 FTU/kg of diet in the finisher phase). In 10, 15, 20, and 30% diets Ca and aP levels were decreased by 10, 15, 20, and 30% of the control.

For each phase, two diets were prepared, one of which contained recommended levels of Ca and aP (summit diet) and the other contained the lowest level of Ca and aP (30%; dilution diet). Then the optimum and dilution diets were thoroughly mixed in the proportions of 50:50 to make the 15%C diet for the grower phase and 67:33 and 33:67 to create 10%C and 20%C diets for the finisher phase, respectively (Table 1). In the dilution diets, sand was used as filler at the expense of dicalcium phosphate and calcium carbonate. The Ca-to-aP ratio was kept constant in all experimental diets.

The calculated and analysed Ca, total phosphorus (tP), and aP levels in all experimental grower and finisher diets are shown in Table 3. Phytase activity determination was conducted for each experimental diet according to Yin, Zheng, and Kang (2007; Table 4). Rearing house temperature was fixed at 32°C in the first three days and decreased by 3°C every week to reach 21°C and then stayed constant until the end of the experimental period. Relative humidity was between 50% and 60% from 11 to 42 d. Light and darkness were set at 18 h light and 6 h dark all over the experiment (11–42 d). Feed and water were provided ad libitum throughout the experiment. Management practices were performed based on the recommendations (Aviagen 2014a).

Growth performance

The birds in each replicate were weighed collectively at 11, 24, and 42 d. The average body weight (AWB), daily weight gain (DWG), and daily feed intake (DFI) were calculated for each pen. Feed intake was calculated by subtracting the

Table 7. Effect of dietary levels of calcium (Ca) and available phosphorus (aP) on jejunal morphology at 42 d of age.

Experimental treatments (Dietary Ca and aP reduction)		jejunal morphology characteristics					
Grower phase	Finisher phase	Villus height (µm)	Villus width (µm)	Crypt depth (µm)	V: C[1]	Muscle thickness (µm)	Villus surface Area[2] (mm^2)
C[3]	C	1140.5	151.20	260.83	4.38	284.33	0.54
	C + phytase[4]	1156.83	151.00	264.50	4.40	282.00	0.55
15%	C	1112.33	154.67	257.17	4.38	251.83	0.54
	10%	1130.5	142.40	257.50	4.42	279.83	0.52
	20%	1144.33	147.83	232.67	4.97	240.67	0.53
	30%	1113.17	137.00	223.50	4.98	252.17	0.48
	C + phytase	1171.5	143.40	245.33	4.81	243.50	0.54
	10% + phytase	1109.83	153.17	247.50	4.51	270.50	0.53
	20% + phytase	1131.33	148.00	240.50	4.73	247.67	0.53
	30% + phytase	1157.83	148.67	246.50	4.70	270.33	0.54
30%	C	1117.83	137.83	241.83	4.64	253.33	0.48
	10%	1110.83	139.17	239.50	4.75	298.83	0.49
	20%	1162.5	139.50	230.83	5.06	303.17	0.51
	30%	1150.5	141.50	226.83	5.07	258.67	0.51
	C + phytase	1111	150.83	240.83	4.62	246.00	0.53
	10% + phytase	1091.67	152.00	245.83	4.46	266.33	0.52
	20% + phytase	1098	148.50	241.67	4.56	302.33	0.51
	30% + phytase	1069.5	147.33	244.83	4.48	248.67	0.49
SEM		40.610	6.693	9.861	0.224	22.620	0.028
P-Value		0.965	0.803	0.210	0.350	0.634	0.912

[1]V: C= villus height to crypt depth ratio. [2]Villus surface area was calculated by (2π) * (villus width/2) * (Villus height). [3]Control diets contained the recommended levels of calcium and available phosphorus based on Ross 308 recommendations (Aviagen 2014b). [4]Phytase was used by 500 FTU/kg of diet in the finisher phase. In 10, 15, 20, and 30% diets Ca and aP levels were decreased by 10, 15, 20, and 30% of the control.

Table 8. Main effects and their interactions of grower regimen, finisher regimen, and phytase on growth performance of broiler chickens.

Treatments		Finisher (25-42 d)				Whole experimental period (11-42 d)		
Main effects		ABW (g/bird)	DWG (g/bird/d)	DFI (g/bird/d)	FCR (g:g)	DWG (g/bird/d)	DFI (g/bird/d)	FCR (g:g)
Grower[1]								
	15%	2350.5	83.49	133.36	1.61	66.55	104.88	1.58
	30%	2341.1	82.86	133.10	1.60	66.14	104.48	1.58
SEM		8.114	0.335	0.621	0.005	0.247	0.463	0.004
Finisher[2]								
	Control[3]	2361.68	84.02	134.08	1.61	66.80	105.34	1.58
	10%	2343.62	83.51	133.33	1.60	66.35	104.43	1.57
	20%	2326.80	82.70	133.26	1.61	65.82	104.34	1.59
	30%	2350.93	82.45	132.24	1.61	66.42	104.60	1.57
SEM		11.475	0.474	0.878	0.007	0.350	0.654	0.006
Phytase[4]								
	-	2345.63	83.22	133.50	1.61	66.32	104.76	1.58
	+	2345.88	83.13	132.96	1.61	66.37	104.60	1.58
SEM		8.114	0.335	0.621	0.005	0.247	0.463	0.004
P-value								
Grower		0.415	0.188	0.765	0.279	0.239	0.534	0.526
Finisher		0.188	0.078	0.530	0.69	0.275	0.697	0.558
Phytase		0.982	0.847	0.538	0.454	0.890	0.798	0.555
Grower × Finisher		0.302	0.845	0.949	0.752	0.528	0.396	0.430
Grower × Phytase		0.891	0.687	0.437	0.150	0.903	0.602	0.332
Finisher × Phytase		0.508	0.594	0.773	0.879	0.653	0.574	0.911
Grower × Finisher × Phytase		0.368	0.322	0.449	0.313	0.264	0.824	0.398
Linear (Ca and aP levels in Grower)		0.416	0.186	0.757	0.267	0.234	0.520	0.516
Linear (Ca and aP levels in Finisher)		0.336	0.042	0.143	0.445	0.285	0.420	0.879
Quadratic (Ca and aP levels in Finisher)		0.066	0.784	0.876	0.985	0.133	0.362	0.565
Linear (Phytase level)		0.983	0.848	0.523	0.445	0.890	0.791	0.546

ABW: average body weight; DWG: daily weight gain; DFI: daily feed intake; FCR: Feed conversion ratio. [1]Grower: reduction of calcium and available phosphorus in the grower phase. [2]Finisher: reduction of calcium and available phosphorus in the finisher phase. [3]Control diets contained 100% Ca and aP recommended by Ross 308 company (Aviagen 2014b).[4]Phytase was used by 500 FTU/kg of diet in the finisher phase (+, diets with phytase; -, diets without phytase). In 10, 15, 20, and 30% diets Ca and aP levels were decreased by 10, 15, 20, and 30% of the control.

Table 9. Main effects and their interactions of grower regimen, finisher regimen, and phytase on jejunal morphology at 42 d of age.

Experimental treatments		jejunal morphology characteristics					
Main effect		Villus height (μm)	Villus width (μm)	Crypt depth (μm)	V: C[1]	Muscle thickness (μm)	Villus surface Area[2] (mm^2)
Grower[3]							
	15%	1133.85	146.89	245.15	4.66	257.06	0.53
	30%	1113.98	144.58	239.02	4.70	272.17	0.51
SEM		14.760	2.376	3.546	0.080	7.596	0.010
Finisher[4]							
	Control[5]	1128.17	146.68	246.29	4.61	248.67	0.52
	10%	1110.71	146.68	247.58	4.53	278.88	0.51
	20%	1134.04	145.96	236.42	4.83	273.46	0.52
	30%	1122.75	143.63	238.04	4.75	257.46	0.51
SEM		20.873	3.360	5.014	0.114	10.742	0.014
Phytase[6]							
	-	1130.25	142.49	240.04	4.76	267.31	0.51
	+	1117.58	148.99	244.13	4.61	261.92	0.52
SEM		14.760	2.376	3.546	0.080	7.596	0.010
P-value							
Grower		0.343	0.502	0.225	0.687	0.163	0.151
Finisher		0.877	0.907	0.287	0.250	0.174	0.822
Phytase		0.545	0.061	0.417	0.196	0.616	0.274
Grower × Finisher		0.986	0.916	0.886	0.961	0.135	0.929
Grower × Phytase		0.151	0.287	0.376	0.078	0.500	0.975
Finisher × Phytase		0.729	0.694	0.403	0.270	0.828	0.941
Grower × Finisher × Phytase		0.759	0.457	0.966	0.974	0.960	0.484
Linear (Ca and aP levels in Grower)		0.321	0.463	0.213	0.686	0.160	0.134
Linear (Ca and aP levels in Finisher)		0.938	0.483	0.103	0.155	0.659	0.473
Quadratic (Ca and aP levels in Finisher)		0.879	0.736	0.979	0.989	0.032	0.821
Linear (Phytase level)		0.528	0.046	0.407	0.192	0.617	0.259

[1]V: C= villus height to crypt depth ratio. [2]Villus surface area was calculated by (2π) * (villus width/2) * (Villus height). [3]Grower: reduction of calcium and available phosphorus in the grower phase. [4]Finisher: reduction of calcium and available phosphorus in the finisher phase. [5]Control diets contained 100% Ca and aP recommended by Ross 308 company (Aviagen 2014b). [6]Phytase was used at a 500 FTU/kg diet in the finisher phase (+, diets with phytase; -, diets without phytase supplementation). In 10, 15, 20 and 30% diets Ca and aP levels were decreased by 10, 15, 20, and 30% of the control diet

Table 10. Effect of dietary levels of calcium (Ca) and available phosphorus (aP) on nutrient apparent total tract retention (ATTR) and tibia traits on 24 d.

	Ca and aP reduction in diets				*P*-Value		
Nutrient ATTR (%)	C[1]	15% C	30% C	SEM	Overall	Linear	Quadratic
Dry matter	72.56	72.13	72.23	0.341	0.652	0.501	0.534
Crude protein	66.48	65.13	67.30	0.889	0.250	0.520	0.127
Calcium	51.05	52.62	53.57	0.651	0.045	0.015	0.702
Total phosphorus	57.39	58.03	60.04	0.756	0.063	0.025	0.475
Phytate phosphorus	39.85	40.12	42.86	0.857	0.047	0.025	0.257
Tibia traits							
Ash (%)	50.09	49.14	47.92	0.594	0.063	0.021	0.858
Calcium (%)	18.18	17.73	16.95	0.312	0.042	0.014	0.677
Phosphorus (%)	8.54	8.37	7.94	0.145	0.028	0.010	0.491
Breaking strength (N)	152.7	138.0	128.6	6.707	0.065	0.022	0.752

[1]Control diets contained the recommended levels of calcium and available phosphorus based on Ross 308 recommendations (Aviagen 2014b).

Table 11. Effect of dietary levels of calcium (Ca) and available phosphorus (aP) on nutrient apparent total tract retention (ATTR) and tibia characteristics at 42 d of age.

Treatments (Dietary Ca and aP reduction)		Nutrient TTAR (%)					tibia characteristics			
Grower phase	Finisher phase	Dry matter	Crude protein	Ca*	Total P	Phytate P	Ash (%)	Ca (%)	P (%)	Breaking strength (N)
C[1]	C	69.28	61.02	44.38^{f}	47.04^{e}	37.25^{g}	49.08^{ab}	17.82^{ab}	8.49^{a}	345.95^{a}
	C + phytase[2]	68.78	61.26	45.51^{ef}	49.21^{cde}	38.71^{efg}	49.11^{a}	17.87^{a}	8.30^{ab}	346.02^{a}
15%	C	68.84	60.91	44.31^{f}	47.07^{e}	38.37^{fg}	48.33^{abc}	17.41^{abc}	8.16^{abc}	299.15^{de}
	10%	68.67	61.06	44.43^{f}	49.25^{cde}	38.64^{efg}	48.12^{abc}	17.09^{abc}	8.18^{ab}	310.87^{cd}
	20%	69.57	63.19	45.37^{ef}	49.60^{c}	40.13^{cdef}	44.94^{fg}	16.77^{bcde}	7.64^{cdef}	279.12^{e}
	30%	68.91	63.25	45.70^{ef}	49.44^{cd}	41.14^{bcde}	44.12^{gh}	15.87^{def}	7.42^{ef}	248.82^{f}
	C + phytase	68.72	60.96	45.98^{ef}	49.77^{c}	38.95^{defg}	48.26^{abc}	17.32^{abc}	8.26^{ab}	329.25^{abc}
	10% + phytase	68.82	60.99	48.73^{bc}	54.08^{ab}	41.94^{bc}	47.96^{abc}	17.19^{abc}	8.19^{ab}	332.07^{abc}
	20% + phytase	69.71	61.57	48.36^{cd}	55.34^{ab}	45.87^{a}	47.86^{bc}	17.21^{abc}	8.22^{ab}	314.15^{bcd}
	30% + phytase	69.79	62.35	49.19^{abc}	54.05^{ab}	45.48^{a}	47.82^{c}	17.04^{abc}	7.88^{bcde}	294.70^{de}
30%	C	69.24	62.33	44.58^{f}	47.12^{de}	37.83^{fg}	47.86^{bc}	17.28^{abc}	8.07^{abcd}	339.23^{abc}
	10%	68.89	60.97	44.59^{f}	49.75^{c}	39.29^{defg}	45.81^{ef}	16.93^{abcd}	7.63^{def}	293.03^{de}
	20%	69.20	61.94	46.25^{def}	49.90^{c}	41.35^{bcd}	44.47^{gh}	15.65^{f}	7.32^{f}	273.30^{ef}
	30%	68.93	62.39	47.33^{cde}	50.57^{c}	41.52^{bcd}	43.42^{h}	15.70^{ef}	7.24^{f}	246.95^{f}
	C + phytase	68.76	60.89	46.39^{def}	49.93^{c}	38.76^{efg}	47.66^{c}	17.09^{abc}	8.08^{abcd}	335.83^{abc}
	10% + phytase	69.70	60.86	49.25^{abc}	53.19^{b}	41.46^{bcd}	47.19^{cd}	16.95^{abcd}	7.85^{bcde}	341.63^{ab}
	20% + phytase	69.78	60.99	51.05^{a}	55.25^{ab}	43.65^{ab}	46.19^{de}	16.77^{bcde}	7.60^{def}	315.67^{bcd}
	30% + phytase	69.22	61.17	50.61^{ab}	56.14^{a}	44.98^{a}	44.69^{fg}	16.34^{cdef}	7.46^{ef}	275.93^{ef}
SEM		0.376	0.843	0.779	0.841	0.921	0.436	0.385	0.187	10.607
P-Value		0.337	0.554	<.0001	<.0001	<.0001	<.0001	0.0008	<.0001	<.0001

*The means of each column with uncommon letters are significantly different (P ≤ 0.05). [1]Control diets contained the recommended levels of calcium and available phosphorus based on Ross 308 recommendations (Aviagen 2014b). [2]Phytase was used at a 500 FTU/kg diet in the finisher phase. In 10, 15, 20, and 30% diets Ca and aP levels were decreased by 10, 15, 20, and 30% of the control.

remaining feed from the offered amount per pen during each study phase. Feed conversion ratio (FCR) was corrected for mortality and expressed as grams of feed consumed by all birds in each pen divided by grams of body weight gain.

Intestinal morphology

Six birds from each treatment were randomly selected and euthanised by cervical dislocation at 24 and 42 d. The entire intestinal tract was removed, and 1 cm segments were taken from the mid-point of the jejunum. The segments were fixed in 10% neutral buffered formalin solution and fixed in paraffin wax later. All histological morphometric analyses were conducted on 5 µm sections, stained with H&E. To study the morphology of the tissue samples, a computer-connected optical microscope (Olympus model B×51 microscope; magnification 100) was used to obtain images of the samples which allowed measurement of villi length and width, crypt depth, muscle layer thickness and villus surface area, which was calculated by:

$$\text{Villus surface area} = (2\pi) \times (\text{average villus width}/2) \times (\text{villus height})]$$

measured using the relevant software (DP2-BSW software).

Apparent total tract retention of crude protein, calcium, total and phytate phosphorus

Eighteen birds at 24 d of age and 12 birds at 42 d of age were selected from each treatment group and placed into six metabolic cages to determine the ATTR of dry matter (DM), crude protein (CP), Ca, tP, and phytate phosphorus (pP). After two days of adaptation, the birds were subjected to a 12 h fasting period. After that, the birds were refed for three days, followed by another 12 h fasting period. Excreta were collected twice daily and stored immediately at – 18°C until subsequent analysis. Excreta collection was carried out from the refeeding period to the end of the latter fasting period. The quantity of consumed feed was recorded for each

Table 12. Main effects and their interactions of grower regimen, finisher regimen, and phytase on nutrient apparent total tract retention (ATTR) and tibia characteristics at 42 d.

Treatments		Nutrient ATTR (%)					Tibia characteristics			
Main effect		Dry matter	Crude protein	Ca	Total P	Phytate P	Ash (%)	Ca (%)	P (%)	Breaking strength (N)
Grower[1]										
	15%	69.08	61.54	46.43	51.00	41.23	47.18	16.99	8.00	301.01
	30%	69.21	61.44	47.50	51.59	41.10	45.91	16.59	7.66	302.70
SEM		0.136	0.296	0.274	0.297	0.330	0.148	0.135	0.061	3.534
Finisher[2]										
	Control[3]	68.89	61.48	45.31	48.47	38.48	48.03	17.28	8.15	325.87
	10%	69.98	60.92	46.68	51.51	40.26	47.27	17.04	7.96	319.40
	20%	69.57	61.86	47.76	52.74	42.75	45.87	16.60	7.69	295.56
	30%	69.16	61.72	48.12	52.47	43.19	45.01	16.24	7.50	266.60
SEM		0.192	0.418	0.388	0.420	0.466	0.209	0.191	0.086	4.997
Phytase[4]										
	-	69.03	61.82	45.32	49.20	39.78	45.88	16.59	7.71	286.31
	+	69.27	61.17	48.62	53.40	42.55	47.20	16.99	7.94	317.40
SEM		0.136	0.296	0.274	0.297	0.330	0.148	0.135	0.061	3.534
P-value										
Grower		0.499	0.809	0.007	0.166	0.786	<.0001	0.039	0.0002	0.737
Finisher		0.070	0.406	<.0001	<.0001	<.0001	<.0001	0.001	<.0001	<.0001
Phytase		0.220	0.120	<.0001	<.0001	<.0001	<.0001	0.039	0.007	<.0001
Grower × Finisher		0.410	0.809	0.403	0.412	0.929	0.112	0.659	0.477	0.093
Grower × Phytase		0.749	0.512	0.389	0.754	0.235	0.191	0.988	0.540	0.696
Finisher × Phytase		0.454	0.725	0.099	0.187	0.064	<.0001	0.144	0.364	0.241
Grower × Finisher × Phytase		0.605	0.700	0.854	0.675	0.515	0.009	0.722	0.717	0.154
Linear (Ca and aP levels in Grower)		0.504	0.805	0.058	0.399	0.850	0.001	0.056	0.001	0.827
Linear (Ca and aP levels in Finisher)		0.100	0.363	0.0001	<.0001	<.0001	<.0001	0.0001	<.0001	<.0001
Quadratic (Ca and aP levels in Finisher)		0.203	0.614	0.346	0.007	0.225	0.877	0.749	0.943	0.067
Linear (Phytase level)		0.224	0.107	<.0001	<.0001	<.0001	0.0005	0.057	0.026	<.0001

[1]Grower: reduction of calcium and available phosphorus in the grower phase. [2]Finisher: reduction of calcium and available phosphorus in the finisher phase. [3]Control diets contained 100% Ca and aP recommended by Ross 308 company (Aviagen 2014b). [4]Phytase was used at 500 FTU/kg diet in the finisher phase (+, diets with phytase; -, diets without phytase supplementation). In 10, 15, 20, and 30% diets Ca and aP levels were decreased by 10, 15, 20, and 30% of the control diet.

metabolic cage. Feed samples and excreta were dried at 55°C for 48 h using a forced air oven and then ground by a blade grinder and weighed to calculate collective DM intake and excretion. All samples were analysed for total nitrogen (TN) via the Kjeldahl method, according to AOAC international (Association of Official Analytical Chemists (AOAC) 2005; Method 2001.11). Then, each sample's CP was calculated by multiplying its TN value by 6.25. To determine dietary and excreta Ca and tP contents, inductively coupled plasma optical emission spectroscopy (ICP-OES; Spectro Arcos System, Germany, model 76004555). A modified model of Gao et al. (2007) was used to measure pP. In this method, the amount of phytic acid is calculated from organic P, assuming that one molecule of phytic acid contains six molecules of P.

Contents of ash, calcium, phosphorus, and breaking strength of tibia

On 24 and 42 d, six birds from each treatment weighing closest to the average weight of that pen were randomly selected and slaughtered, respectively. The left tibia from each slaughtered male bird was removed to evaluate the percentage of ash, Ca, P, and BS at 24 and 42 d. After separating the meat and soft tissues from each tibia, it was weighed, and the Instron Universal Testing Machine (Model H5KS, Tinius Olsen Company) was used to determine its BS. The BS was calculated using the software Q Mat. Tibias were defatted by soaking in ethyl alcohol for 48 h and again in ethyl ether for 48 h, then dried to a constant weight using a drying oven at 105°C for 24 h. Then, they were ashed in a muffle furnace at 550°C for 12 h (Imari *et al.*, 2020b). The remaining ash was used to determine tibia Ca and P contents by employing the ICP-OES method. (Model H5KS, Tinius Olsen Company).

Statistical analyses

This experiment was carried out during the grower phase in a completely randomised design with three treatments including, C with 12 replicates and diets with 15 and 30% reduction in Ca and aP each with 48 replicates. The analysis of variance for the finisher phase (25–42 d) was carried out as a completely randomised design with 18 treatments of 6 replicates each. Then, after excluding the C treatment, analysis of variance for the finisher phase was also carried out in a completely randomised design as a $2 \times 4 \times 2$ (grower regimen × finisher regimen × phytase) factorial arrangement with 16 treatments of 6 replicates each by the GLM procedure of SAS 9.4 (2012) software. All data were normalised before statistical analysis. Orthogonal polynomial contrasts (linear and quadratic) were performed in response to reduced levels of Ca and aP in the diets for each feeding phase (grower and finisher).

Results

Growth performance and intestinal morphology

The results of AWB, DWG, DFI, and FCR for the grower phase and jejunal morphology at 24 d are shown in Table 5.

The AWB, DWG, DFI, and FCR and jejunal morphology at 42 d are shown in Tables 6 and 7, respectively.

The main effects and their interactions of grower and finisher regimen, and phytase on growth performance and jejunal morphology are also presented in Tables 8 and 9, respectively. The treatments did not significantly affect the growth performance and jejunal morphology during the grower (11–24 d) and finisher (25–42 d) phases.

Nutrient apparent total tract retention and tibia characteristics

The effect of reducing levels of Ca and aP in the diet on nutrient ATTR and tibial characteristics on 24 and 42 d are shown in Tables 10 and 11, respectively. Reducing dietary Ca and aP during the grower and finisher phase did not have any significant effect on ATTR of DM or CP at 24 or 42 days of age ($P > 0.05$). At 24 d, the ATTR of Ca and aP linearly increased with decreasing levels of Ca and aP ($P < 0.05$). In addition, by reducing dietary Ca and aP in the grower diets, contents of Ca and P of the tibia were linearly decreased ($P < 0.05$).

On 42 d, the ATTR of Ca, tP, and pP became greater with the reduction in dietary Ca and aP levels ($P < 0.05$); The lowest ATTR was observed in birds fed the C diet. Concerning the ATTR of Ca, there were significant differences between C and 10, 20, and 30%C + phytase diets with 15% or 30% reduction in Ca and aP in the grower phase. In addition, a significant difference was observed between C and 30%C diets with a 30% reduction of Ca and aP in the grower phase. Regarding the ATTR of tP, there were significant differences between C and C + phytase, 10, 20 and 30%C + phytase within 15% or 30% reduction of Ca and aP in the grower phase and significant differences were observed between C and 20, 30% and between C and 10, 20, 30% within 15% or 30% reduction of Ca and aP in grower phase, respectively. Significant differences were observed between C and 20%C and 30%C with the 15% or 30% reduction of Ca and aP in the grower phase. Moreover, there were significant differences between C, 10%C, 20%C and 30%C + phytase with the 15% or 30% reduction of Ca and aP in the grower phase.

At 42 d of age, tibia ash, Ca, P, and BS were significantly decreased by a reduction in dietary Ca and aP ($P < 0.05$). Significant differences were observed between C and 20, 30%, and 30% + phytase within a 15% reduction of Ca and aP in the grower phase. Furthermore, there were significant differences between C and 10%C, 20%C, 30%C, C + phytase, 10% C + phytase, 20%C + phytase, and 30% + phytase within 30% reduction of Ca and aP in the grower phase. In relation to tibia Ca (%), significant differences were observed between C and 30%C and between C and 20%C, 30%C within 15% or 30% reduction of Ca and aP in the grower phase, respectively. In addition, there were significant differences between C and 30%C + phytase within a 30% reduction of Ca and aP in the grower phase. Tibia P content showed significant differences between C and 20, 30%, 30% + phytase with a 15% reduction of Ca and aP in the grower phase, and there were significant differences between C and 10%C, 20%C, 30%C, 10%C + phytase, 20%C + phytase, and 30%C + phytase with a 30% reduction of Ca and aP in the grower phase. In relation to BS of the tibia, significant differences were observed between C and 10%C, 20%C, 30%C, 20%C+ phytase and 30%C + phytase with the 15% reduction of Ca and aP in the grower phase and there were significant differences between C and 10%C, 20%C, 30%C, 20%C + phytase and 30%C + phytase within 30% reduction of Ca and aP in the grower phase.

The main effects and their interactions due to grower and finisher regimen, and phytase on nutrient ATTR and tibia characteristics at 42 d are shown in Table 12. On 42 d, the main effects of the finisher phase and phytase significantly affected ($P < 0.05$) the ATTR of Ca, tP, and pP (Table 12). By reducing the levels of Ca and aP in the finisher diets, ATTR of Ca, tP, and pP linearly increased ($P < 0.05$). Regarding the main effect of phytase, ATTR of Ca, tP, and pP were linearly ($P < 0.05$) raised by supplementation at 500 FTU/kg diet. The main effect of the grower phase on the P content of the tibia was significant; whereby reducing dietary Ca and aP levels, the P content of the tibia was linearly decreased ($P < 0.05$). The main effect of the finisher phase on the Ca and P contents and BS of the tibia was significant ($P < 0.05$(. Tibia Ca and P content and BS were linearly ($P < 0.05$) decreased with reducing levels of Ca and aP in the finisher diet. Regarding the main effect of phytase on tibia P content and BS, phytase supplementation during the finisher phase linearly ($P < 0.05$) increased P contents and BS of the tibia. On 42 d, the three-way interaction effect of grower regimen, finisher regimen, and phytase supplementation on the percentage of tibia ash was significant ($P < 0.05$); so, by decreasing the levels of Ca and aP in the finisher diets (without phytase supplementation) within 15 or 30% reduction of Ca and aP in grower phase, t tibia ash linearly decreased ($P < 0.05$). Furthermore, by decreasing Ca and aP in the finisher diets (plus phytase) with a 30% reduction in Ca and aP in the grower phase, tibial ash was linearly ($P < 0.05$) decreased (Figure 1).

Discussion

In this experiment, restricting Ca and aP in the diet by 30% during the grower and finisher period did not significantly affect the growth performance of broilers. According to Gautier, Walk, and Dilger (2017), a balanced Ca: aP ratio is essential for the formulation of broiler diets. A meta-analysis study predicted that, during the grower phase, 0.45% and 0.44% P with 1.00% Ca were required for maximum feed intake and weight gain, respectively, while similar growth performance with 0.34 and 0.30% P with a 0.60% Ca could be achieved. These researchers suggested that, at lower levels of P, a lower Ca:P ratio was needed to ensure maximum response (Letourneau-Montminy et al. 2010). Rao et al. (2006) reported that birds fed low Ca levels (from 0.90% to 0.60%) throughout the grower phase (1–42 d) achieved better growth performance with the lowest P levels (0.30% and 0.35%) at 28 and 42 d. In agreement with the current study, Rousseau et al. (2012) did not observe any significant difference in growth performance when birds were fed a diet with reduced Ca and P levels from 22 to 35 d. It has been suggested that the adaptation of birds (for instance, probably enhancement of nutrient digestibility) to Ca and P deficiencies at older ages can

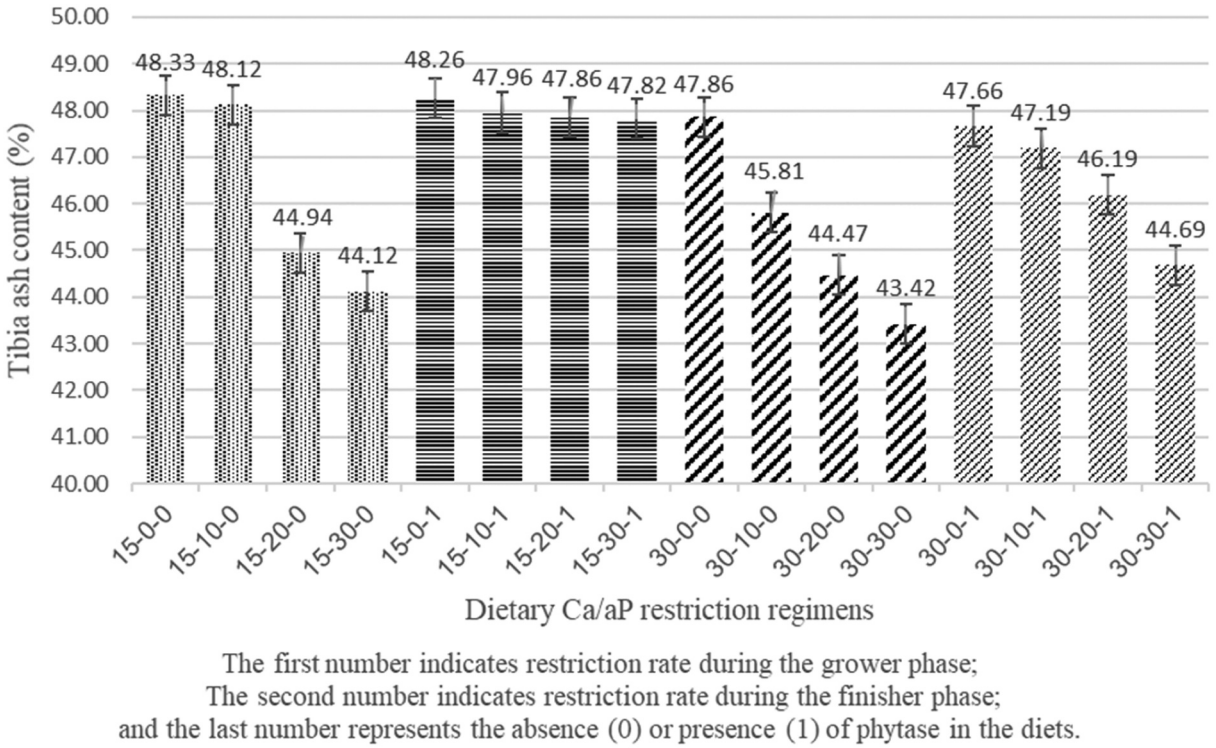

Figure 1. The interaction effect among grower regimen, finisher regimen, and phytase supplementation on tibia ash (%) at 42 d.

eliminate the adverse impacts of these shortages (Yan et al. 2005).

Examination of the morphology of the small intestine can be used as an indicator of intestinal health and integrity. In the current experiment, decreased dietary Ca and aP levels during grower and finisher phases did not significantly affect the jejunal morphology of broilers. In agreement with these findings, Imari *et al*. (2020a) reported that, with decreasing dietary levels of Ca and P up to 30% at 10 and 42 d of age, no significant effect was observed on jejunal morphology, in terms of villus height, crypt depth and villus height-to-crypt depth ratio). Oikeh et al. (2019) studied the interactions between dietary Ca and P levels and vitamin D sources on bone mineralisation, performance and intestinal morphology of coccidia-infected birds. They observed that the birds fed diets containing deficient in Ca and P showed a similar villus height, crypt depth, and villus height to crypt depth ratio in three sections of the small intestine (duodenum, jejunum, and ileum), when compared with birds fed a diet containing adequate Ca and P.

In the present experiment, by reducing the levels of Ca and aP in the diets, the ATTR of Ca, tP, and pP were significantly increased. Rousseau et al. (2016) reported that, with a finisher diet containing 0.35% P, the ATTR of P was increased by decreasing Ca levels from 0.90 to 0.70%. Plumstead et al. (2008) showed that, at a constant level of 0.34% of dietary AP, the apparent pre-caecal digestibility of Ca, tP, and Pp were linearly increased by decreasing dietary Ca levels from 1.16 to 0.47%. Akter, Graham, and Iji (2017) stated that enhancement in the concentration of Ca in a diet with low P led to a decrease in the ileal digestibility of Ca and P. Blahos, Care, and Sommerville (1987) reported an increase in duodenal and ileal P uptake in broilers fed low-Ca diets for two weeks. They observed a slight increase in duodenal and ileal P uptake in chickens fed low-P diets. These adaptations were hypothesised to limit P or Ca due to elevated levels of 1, 25 dihydroxy D3, and duodenal calbindin. It has been stated that broilers fed 1.25% Ca had higher crude protein, ash and P digestibility compared to those fed a diet containing 1.5% Ca. Birds consuming 1.25% Ca diet had higher digestibility of ash, Ca and P compared to those fed 1% Ca (Abdulla et al. 2016). Liu, Chen, and Adeola (2013) stated that the digestibility of P was not affected by dietary P concentration when it met requirements. Oliveira et al. (2018) reported no interaction between phytase and Ca: aP ratios for the apparent ileal digestibility of P in the period from 22 to 33 d. Increasing dietary Ca *via* the consumption of limestone can increase the pH of the gastrointestinal tract due to its high acid-binding capacity (Selle, Cowieson, and Ravindran 2009). Plumstead et al. (2008) found a decline in phytase efficiency when Ca increased from 0.47 to 1.16% in the diet, which reduced the ileal digestibility of pP. In the current experiment, ATTR of Ca, tP, and pP were improved. It was concluded that a reduction in dietary Ca levels probably had no remarkable effect on phytase proficiency. On the other hand, improved ATTR could be due to the bird's adaptation to dietary Ca and P deficiency. Consequently, the bird's performance was not likely affected by reduced dietary Ca and P levels.

In the current study, reducing dietary Ca and aP caused Ca and P, and BS of the tibia to linearly decrease. Imari *et al.* (2020b) showed that lower dietary Ca and aP levels led to a decrease in femur BS and contents of ash, Ca and P contents in the tibia. They stated that this reduction was further observed in chickens fed post-starter diets (11–42 d) that had a higher decrease in Ca and aP. Broilers fed diets with a 10% Ca and aP reduction in the post-starter period,

irrespective of the Ca and aP restrictions in the starter phase, had no significant difference with the control group. They observed that chickens fed diets containing 20 and 30% Ca and aP reduction in the post-starter period could not compensate for the deficiency of these minerals. They concluded that the dietary Ca and aP levels could be reduced by 30% in the starter phase; however, the restriction of these minerals in the post-starter period by more than 10% was not advisable (Imari *et al.*, 2020b). In agreement with the present experiment, Mondal, Panda, and Biswas (2007) reported that the tibia ash, Ca, and P decreased in the broilers who consumed low-P diets (0.30% aP) compared with the control group (0.46% aP). Similarly, Wilkinson et al. (2014) showed that the tibia ash was reduced by changing dietary P from 0.35 to 0.25%. In the study by Gautier, Walk, and Dilger (2017), tibia BS and ash were significantly higher in birds fed 0.4 or 0.6% Ca inclusion compared those birds fed diets containing 1.6% Ca. Rao et al. (2006) reported that tibia BS and ash in birds fed the lowest levels of Ca and P (0.6, 0.3%, respectively) were similar to those fed diets with the highest levels of these two minerals (0.9, 0.54%, respectively). Reduction of dietary Ca, especially in low P diets, limited tibial ash weight, ash content and BS at 21 d in birds fed 0.6% Ca and 0.3% aP compared to those fed 1.0% Ca and 0.45% P, while at 35 d, no significant differences in tibial characteristics were observed between treatments (Rousseau et al. 2016). Bavaresco et al. (2020) concluded that the use of phytase in broiler diets with a 0.16-unit reduction in Ca and aP, regardless of the Ca: aP ratio, improved bone characteristics (Ca, and P contents and BS). In the current study, phytase supplementation at a 500 FTU/kg diet increased the Ca and P contents and BS of the tibia. High levels of Ca in the diet can decrease phytase activity (Lei et al. 1994) because it acts as a chelating agent for most nutrients released by phytase and significantly limits nutrient digestibility and performance (Coelho and Kornegay 1996).

In conclusion, Ca and aP levels in the grower and finisher diets can be reduced up to 30% of the recommendations without affecting growth performance and intestinal morphology. By reducing dietary Ca and aP levels in grower and finisher diets, ATTR of Ca, aP and pP were improved. However, a decrease in dietary calcium and aP had adverse effects on the tibia, which reduced its ash content. Therefore, reducing Ca and aP in the diet should be done with caution so as not to endanger the skeletal health and well-being of the birds. Using 500 FTU/kg phytase can improve bone traits to some extent.

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