The Effect of Feeding Calcium- and Phosphorus-Deficient Diets to Broiler Chickens During the Starting and Growing-Finishing Phases on Carcass Quality

J. P. Driver,[1] G. M. Pesti, R. I. Bakalli, and H. M. Edwards Jr.

Department of Poultry Science, University of Georgia, Athens 30602

ABSTRACT There is considerable data on the effect of reducing inorganic Ca and P in broiler finisher diets on carcass quality. However, there is limited information on the effect of reducing dietary Ca and P during the different phases of growout. Two experiments were conducted from 0 to 35 d in floor pens. In both experiments, at least 4 replicates per treatment (50 chicks per replicate) were used. Corn-soybean meal and soybean oil-based diets deficient in Ca and P were fed. During the starter phase (ST), from 0 to 18 d, chicks were fed a 23% CP diet containing 0.60% Ca and 0.47% total P (tP). During the grower-finisher phase (GF), from 19 to 35 d, birds were fed a 19% CP diet containing 0.30% Ca and 0.37% tP. A combination of 1,000 phytase units/kg of Natuphos phytase and 5 μg/kg of 1α-hydroxycholecalciferol (P + 1α) was supplemented to some of the feed during the ST and GF. Diets containing adequate Ca and P were also fed during the ST (0.90% Ca and 0.68% tP) and GF (0.80% Ca and 0.67% tP). The level of tibia ash and the incidence of bone disease were measured at 18 and 35 d. At the end of the experiments, birds were processed and evaluated for muscle hemorrhages and broken bones. In both experiments, broilers fed diets that were not P + 1α supplemented demonstrated poor bone mineralization, considerable leg problems, and a high incidence of broken bones after processing. Broilers fed P + 1α throughout had more broken clavicles and femurs compared with birds fed the adequate diets. Day-18 tibia ash was significantly correlated to broken tibias and femurs during processing. Day-35 tibia ash was better correlated to bloody breast meat than to broken bones. It is concluded that carcass quality depends on the levels of Ca and P fed and the age of the bird. Tibia ash, traditionally used as an indication of bone strength, was better correlated to the incidence of bloody breasts.

Key words: calcium, phosphorus, broiler, processing, carcass quality

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INTRODUCTION

Although it is now accepted that low levels of Ca and P can be fed during the finisher phase without compromising performance (Skinner et al., 1992a,b; Skinner and Waldroup, 1992; Chen and Moran, 1994, 1995; Dhandu and Angel, 2003), reducing the Ca and P in the diet can cause broken bones and bloody meat during processing of the carcass (Chen and Moran, 1995). This is very significant due to the increased importance of selling cut-up chicken parts in which the emphasis is no longer only on yield but also on characteristics such as bloody breast meat and broken bones (Gregory and Wilkins, 1990). Broken bones, especially fractured clavicle bones, are important, as fragments that find their way into the meat must be removed at great expense. Hemorrhages in the meat are another major quality defect, which can lead to downgrading of the broiler carcass.

Most work conducted to evaluate the effect of dietary Ca and P on carcass quality has concentrated on skeletal problems, and the majority of these experiments have been conducted using broilers fed Ca- and P-adequate diets until at least 32 d of age (Skinner et al., 1992a,b; Skinner and Waldroup, 1992; Chen and Moran, 1994; Dhandu and Angel, 2003). There is a paucity of data on the effect of reducing dietary Ca and P during early life on carcass quality characteristics, because few studies have evaluated characteristics other than bone ash and bone breaking strength.

Two experiments were conducted in the current work to determine how various carcass quality characteristics are influenced when broiler chicks are fed a suboptimal level of dietary Ca and P during the early and late periods of the growth cycle. The deficient diets were also supplemented with various combinations of 1α-hydroxycholecalciferol (**1α-OHD$_3$**) and Natuphos phytase (BASF Corp., Wyandotte MI) to assess whether increased Ca and P absorption could offset deficiencies of these minerals in the diet.

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[1]Corresponding author: jdriver65@hotmail.com

MATERIALS AND METHODS

General Procedures

Experiments 1 and 2 began in December and April, respectively. The broiler house used for both experiments was equipped with space heaters and evaporative cooling. These maintained target temperatures of 30, 29, 27, 26, 24, and 23°C for d 0 to 3, 4 to 6, 7 to 12, 13 to 15, 16 to 22, and 23 to 35, respectively. High and low temperatures were recorded daily and did not vary from targets by more than 2°C. Stirring fans kept temperatures uniform within each room of 24 pens. In Experiment 2, with 2 rooms of 24 pens, the room-to-room temperature variation never exceeded 2°C. The broiler house was completely enclosed and lighting was provided by incandescent bulbs to prevent birds from being exposed to ultraviolet radiation and synthesizing their own vitamin D, the precursor to 1,25-dihydroxycholecalciferol, a biological analogue of 1α-OHD$_3$. Photoperiods were 24L:0D in Experiment 1 and 23L:1D in Experiment 2. All diets were provided for ad libitum consumption. The University of Georgia Animal Care and Use Committee approved all bird-handling protocols.

Experiment 1

Five dietary feeding regimens (treatments) were fed in which each regimen consisted of starter phase (**ST**) diet from 0 to 18 d and a grower-finisher phase (**GF**) diet from 19 to 35 d.

All diets (Table 1) contained 1,100 IU/kg of vitamin D$_3$ and were fed in mash form. Two separate basal Ca- and P-deficient diets were mixed (Table 1). The first, fed during the ST, was formulated to contain 0.60% Ca and 0.24% nonphytin P [0.47% total P (**tP**)], and the second, fed during the GF, was formulated to contain 0.30% Ca and 0.13% nonphytin P (0.37% tP). Each deficient diet was mixed with and without both 1,000 phytase units (**FTU**)/kg of Natuphos phytase (analyzed activity was 1,701 FTU/kg; analysis performed in vitro from feed by supplier, BASF Corp.) and 5 μg/kg of 1α-OHD$_3$. One feeding regimen served as a positive control and consisted of 2 Ca-and P-adequate diets (Table 1). Neither of the adequate diets were supplemented with phytase or 1α-OHD$_3$. These diets were fed to 1,200 Cobb × Cobb broiler chicks of mixed sex that were randomly allocated, 50 per pen, to 24 floor pens (119 × 300 cm) covered with pine shavings. Each pen was considered a replicate.

A randomized block design was used, in which the 24 pens in the house were divided into 4 blocks of 6 pens each. Each block contained 1 replicate from each feeding regimen, except for the positive control regimen, which appeared twice in each block. Therefore, 4 replicates per treatment were used, with the exception of the control, which had 8.

At 18 and 35 d, 10 birds per pen were selected at random by sex. They were killed by CO_2 asphyxiation, and their left tibias were removed for bone ash determination on a dry fat-free basis (Association of Official Analytical Chemists, 1995). Their right tibias were used to determine the incidence and severity of Ca rickets (Long et al., 1984a), P rickets (Long et al., 1984b), and tibial dyschondroplasia using the 0 to 3 scoring system, as described by Edwards and Veltmann (1983). Five male and 5 female birds were randomly selected from each pen on d 35 for processing on d 36. Feed was withdrawn from the birds at approximately 12 h before slaughter, and the birds were tagged and placed together in 1 of 4 floor pens until they were transported to processing using plastic crates. The birds were slaughtered at The University of Georgia poultry processing facility, located approximately 300 yards away from the broiler houses. The birds were stunned with 14 V, 60 Hz of alternating current for 9 s using a commercial stunner (model SF-7001, Simons Engineering Co., Dallas, GA) and then killed by manually cutting the carotid artery and jugular vein on the side of the neck. After exsanguination, the birds were scalded at 54°C for 120 s and picked for 30 s. The scalder (model SS300CF, Cantrell Machine Co. Inc., Gainesville, GA), picker (model CPF-60, Cantrell Machine Co. Inc), and eviscerator (model Mark 4, Cantrell Machine Co. Inc) in the automated line system imposed most of the physical stresses normally encountered under commercial processing conditions, with the exception that the warm eviscerated carcasses were static-chilled in slush ice for 4 h rather than by convective agitation. Each carcass was examined for broken tibias after the eviscerator. Carcasses were then chilled and individually wrapped in plastic bags and placed in a cool room (5°C) until they were dissected on d 37. One person dissected all the carcasses following common practices: Carcasses were suspended on plastic cones, after which the breast skin was removed and breast meat was cut from the carcass, along with the wings. The wings were subsequently removed from each breast. Both femurs were dislocated to remove the thighs from the frame at the joint. Carcasses were evaluated for broken clavicles, bloody breast meat (both the pectoralis major and pectoralis minor muscles), and broken femur shanks. The same person (who was unaware of the experimental treatments) made all subjective measurements.

Experiment 2

In Experiment 2, 2,400 broiler chickens of mixed sex were used and randomly allocated, 50 per pen, to 48 floor pens. Each pen was considered a replicate. The basal diet formulations were identical to those in Experiment 1 (Table 1). There were 3 ST diets (0.60% Ca, 0.47% tP; 0.60% Ca, 0.47% tP + 1,000 FTU/kg of Natuphos phytase + 1α-OHD$_3$; 0.90% Ca, 0.68% tP) and 4 GF diets (0.30% Ca, 0.37% tP; 0.30% Ca, 0.37% tP + phytase; 0.30% Ca, 0.37% tP + phytase + 1α-OHD$_3$; 0.80% Ca, 0.67% tP) fed in a factorial arrangement, making 12 regimens. Each room contained 2 blocks of randomly assigned treatment regimens. Each of the 12 treatments appeared once in each block, making up 4 replicates per treatment. All other details regarding Experiment 2 were identical to

Table 1. Composition of basal diets, Experiments 1 and 2

Ingredient	Starter				Grower-finisher			
	Deficient		Adequate		Deficient		Adequate	
	(%)							
Ground yellow corn	52.72		52.72		65.44		65.44	
Soybean meal (dehulled)	37.63		37.63		27.17		27.17	
Soybean oil	5.78		5.78		3.87		3.87	
Iodized NaCl	0.45		0.45		0.33		0.33	
DL-Met	0.19		0.19		0.11		0.11	
Vitamin premix[1]	0.25		0.25		0.25		0.25	
Trace mineral premix[2]	0.08		0.08		0.08		0.08	
Coccidiostat[3]	0.08		0.08		0.08		0.08	
Bacitracin BMD-60[4]	0.05		0.05		0.05		0.05	
$CuSO_4 \cdot 5H_2O$	0.05		0.05		0.05		0.05	
Sand	1.28		0.00		2.06		0.00	
Dicalcium phosphate	0.52		1.64		0.00		1.71	
Limestone	0.92		1.08		0.51		0.86	
Calculated composition[5]								
ME, kcal/kg	3,200		3,200		3,200		3,200	
CP, %	23.00		23.00		19.00		19.00	
Ca, %	0.60		0.90		0.30		0.80	
tP,[6] %	0.47		0.68		0.37		0.67	
nPP,[7] %	0.24		0.45		0.13		0.45	
Analyzed composition	Exp[8] 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2
Ca, %	0.80	0.55	0.96	0.92	0.38	0.30	0.91	0.07
tP, %	0.48	0.47	0.69	0.67	0.33	0.34	0.64	0.65

[1]Vitamin mix provided the following (per kg of diet): thiamin-mononitrate, 2.4 mg; nicotinic acid, 44 mg; riboflavin, 4.4 mg; D-Ca pantothenate, 12 mg; vitamin B_{12} (cobalamin), 12.0 μg; pyridoxine-HCl, 2.7 mg; D-biotin, 0.11 mg; folic acid, 0.55 mg; menadione Na bisulfate complex, 3.34 mg; choline chloride, 220 mg; cholecalciferol, 1,100 IU; transretinyl acetate, 5,500 IU; all-*rac*-tocopherol acetate, 11 IU; and ethoxyquin, 150 mg.

[2]Trace mineral mix provides the following (per kg of diet): Mn ($MnSO_4$ H_2O), 60 mg; Fe ($FeSO_4$ $7H_2O$), 30 mg; Zn (ZnO), 50 mg; Cu ($CuSO_4$ $5H_2O$), 5 mg; and I (ethylene diamine dihydroiodide), 1.5 mg.

[3]Aviax, Phibro Animal Health, Fairfield, NJ.

[4]Alpharma Inc., Fort Lee, NJ.

[5]Calculated from NRC (1994).

[6]tP = total P.

[7]nPP = nonphytin P.

[8]Exp = experiment.

Experiment 1, with the exception that processing and carcass dissection were completed over 4 d (2 blocks each d) instead of 2, and 2 people dissected the carcasses. In addition, the incidence of valgus (knock-kneed) deformation of the intertarsal joint, as described by Julian (1984), was recorded for those birds removed on d 18 and 35.

Statistical Analysis

An ANOVA was performed on all data for both experiments using the GLM procedure of SAS (SAS Institute, 2001) appropriate for a randomized block design. The pen mean was the experimental unit. Treatment means were compared using Duncan's multiple range test (Duncan, 1955). Pearson correlation coefficients were generated from 18- to 35-d tibia ash values and several variables, including the incidence of P rickets, Ca rickets, and tibial dyschondroplasia and different carcass parameters.

RESULTS

Performance data, as well as bone ash and bone disease data, for broilers in Experiments 1 and 2 are reported in Driver et al. (2005).

Experiment 1

Carcasses of chickens that were fed the Ca- and P-deficient diets throughout the experiment (regimen 1) had an increased incidence of broken tibias (at the $P = 0.07$ level) after evisceration compared with carcasses from regimens 4 and 5, which had no broken tibias (Table 2). Only 2 out of 80 tibias were broken in the regimen when the phytase and 1α-OHD_3 supplements were fed exclusively during the ST (regimen 2).

No significant difference ($P > 0.05$) in the incidence of femurs with broken shanks was found among regimens, but regimen 5 was the only regimen in which no broken femurs were observed (Table 2).

In general, the addition of supplements to the deficient diets did little to reduce the incidence of broken clavicles observed after the carcasses were dissected, irrespective of which phase the supplements were fed (Table 3). There was also no decrease in the incidence of bloody pectoralis major and pectoralis minor muscles when the combination of supplements was fed. Carcasses from birds fed the Ca- and P-adequate diets (regimen 5) had significantly ($P < 0.05$) lower numbers of broken clavicles compared

Table 2. Effect of phytase and 1α-hydroxycholecalciferol (1α-OHD$_3$) supplementation (P + 1α) on broken tibia and femur incidence of broiler chickens (0 to 35 d) after carcass evisceration and separation, Experiment 1

								Percentage	
		Starter (0 to 18 d)			Grower-finisher (19 to 35 d)				
Regimen	Repititions	Ca	tP[1]	Supplement[2]	Ca	tP[1]	Supplement[2]	Broken tibia incidence	Broken femur incidence
1	4	0.60	0.47	—	0.30	0.37	—	16.3 ± 10.7[a]	6.3 ± 3.8[a]
2	4	0.60	0.47	P + 1α	0.30	0.37	—	2.5 ± 2.5[ab]	3.8 ± 3.8[a]
3	4	0.60	0.47	—	0.30	0.37	P + 1α	12.5 ± 4.3[ab]	6.3 ± 1.3[a]
4	4	0.60	0.47	P + 1α	0.30	0.37	P + 1α	0.0 ± 0.0[b]	1.3 ± 1.3[a]
5	8	0.90	0.68	—	0.80	0.67	—	0.0 ± 0.0[b]	0.0 ± 0.0[a]
							ANOVA		
							R^2	0.42	0.47
							Source	df	*P*-value
							Regimen	4	0.0705	0.0954
							Block	3	0.8310	0.2235
							Error	16		

[a,b]Values within variables with no common superscripts differ ($P < 0.05$) when tested with Duncan's multiple range test following ANOVA.

[1]tP = total P.

[2]P + 1α represents 1,000 phytase units/kg of Natuphos 5000 (BASF Corp., Wyandotte MI) plus 5μg/kg of 1α-OHD$_3$.

with the other treatment groups, with the exception of regimen 3.

Experiment 2

The incidence of broken tibias after evisceration and broken femurs after carcass dissection were influenced by both the ST and GF diets at the $P = 0.08$ level (Table 4). Although few significant differences were observed among treatments for these 2 variables, due to a high degree of bird-to-bird variation, it was clear that birds fed the unsupplemented diet during the ST had a higher incidence of broken tibias and femurs compared with birds fed the supplemented or adequate diets. Feeding the adequate diet during the GF consistently reduced the incidence of broken tibias and femurs (regimens 4, 8, and 12; Table 4). Feeding the supplemented diets during the GF usually did not produce the same improvements.

The incidence of broken clavicles and bloody breast muscles after carcass dissection was not influenced by which diet was fed during the ST ($P > 0.14$, analysis not included; Table 5). However, these variables were significantly affected by the type of diet that was fed during the GF ($P < 0.03$, analysis not included). Feeding the adequate diet during the GF consistently reduced the incidence of broken clavicles and bloody breast meat yields (regimens 1 vs. 4, 5 vs. 8, and 9 vs. 12; Table 5). Feeding the supplements with the deficient diet during the GF did not reduce the incidence of clavicular breakage or muscle bleeding compared with when the deficient

Table 3. Effect of phytase and 1α-hydroxycholecalciferol (1α-OHD$_3$; P + 1α) supplementation on broken clavicle incidence and breast meat quality of broiler chickens (0 to 35 d) after carcass separation, Experiment 1

									Percentage	
		Starter (0 to 18 d)			Grower-finisher (19 to 35 d)					
Regimen	Repititions	Ca	tP[1]	Supplement[2]	Ca	tP[1]	Supplement[2]	Broken clavicle incidence	Bloody pectoralis minor incidence	Bloody pectoralis major incidence
1	4	0.60	0.47	—	0.30	0.37	—	50.0 ± 10.8[a]	46.3 ± 11.6[a]	43.8 ± 15.6[a]
2	4	0.60	0.47	P + 1α	0.30	0.37	—	50.0 ± 4.1[a]	50.0 ± 6.1[a]	45.0 ± 14.6[a]
3	4	0.60	0.47	—	0.30	0.37	P + 1α	30.0 ± 9.1[ab]	33.8 ± 6.6[ab]	52.5 ± 19.3[a]
4	4	0.60	0.47	P + 1α	0.30	0.37	P + 1α	50.0 ± 4.1[a]	30.0 ± 18.2[ab]	38.8 ± 18.2[ab]
5	8	0.90	0.68	—	0.80	0.67	—	20.0 ± 4.2[b]	13.8 ± 5.0[b]	25.0 ± 8.2[b]
							ANOVA			
							R^2	0.65	0.75	0.89
							Source	df	*P*-value	
							Regimen	4	0.0033	0.0024	0.0122
							Block	3	0.2342	0.0037	<0.0001
							Error	16		

[a,b]Values within variables with no common superscripts differ ($P < 0.05$) when tested with Duncan's multiple range test following ANOVA.

[1]tP = total P.

[2]P + 1α represents 1,000 phytase units/kg of Natuphos 5000 (BASF Corp., Wyandotte MI) plus 5 μg/kg of 1α-OHD$_3$.

Table 4. Effect of phytase and 1α-hydroxycholecalciferol (1α-OHD$_3$; P + 1α) supplementation on broken tibia and femur incidences of broiler chickens after carcass evisceration and separation (0 to 35 d), Experiment 2

		Starter (0 to 18 d)			Grower-finisher (19 to 35 d)			Percentage	
Regimen	Repititions	Ca	tP[1]	Supplement[2]	Ca	tP[1]	Supplement[2]	Broken tibia incidence	Broken femur incidence
1	4	0.60	0.47	—	0.30	0.37	—	25.0 ± 10.6[a]	6.3 ± 3.8[ab]
2	4	0.60	0.47	—	0.30	0.37	P	22.5 ± 10.5[ab]	10.0 ± 6.1[a]
3	4	0.60	0.47	—	0.30	0.37	P + 1α	16.3 ± 6.9[ab]	2.5 ± 2.5[b]
4	4	0.60	0.47	—	0.80	0.67	—	5.0 ± 3.5[ab]	1.3 ± 1.3[b]
5	4	0.60	0.47	P + 1α	0.30	0.37	—	8.9 ± 4.2[ab]	3.8 ± 1.3[ab]
6	4	0.60	0.47	P + 1α	0.30	0.37	P	3.9 ± 2.4[b]	6.3 ± 3.8[ab]
7	4	0.60	0.47	P + 1α	0.30	0.37	P + 1α	6.3 ± 2.4[ab]	0.0 ± 0.0[b]
8	4	0.60	0.47	P + 1α	0.80	0.67	—	2.5 ± 2.5[b]	0.0 ± 0.0[b]
9	4	0.90	0.68	—	0.30	0.37	—	11.3 ± 5.5[ab]	2.5 ± 1.4[b]
10	4	0.90	0.68	—	0.30	0.37	P	8.8 ± 5.9[ab]	1.3 ± 1.3[b]
11	4	0.90	0.68	—	0.30	0.37	P + 1α	3.8 ± 2.4[ab]	1.3 ± 1.3[b]
12	4	0.90	0.68	—	0.80	0.67	—	2.5 ± 2.5[b]	0.0 ± 0.0[b]

ANOVA

R^2		0.43	0.55
Source	df	*P*-value	
Regimen	11	0.0802	0.0522
Block	3	0.2218	0.0021
Error	33		

[a,b]Values within variables with no common superscripts differ ($P < 0.05$) when tested with Duncan's multiple range test following ANOVA.

[1]tP = total P.

[2]P + 1α represents 1,000 phytase units (FTU)/kg Natuphos 5000 (BASF Corp., Wyandotte MI) plus 5μg/kg of 1α-OHD$_3$. P represents 1,000 FTU/kg of Natuphos 5000.

diet was fed without supplementation, except when the chickens were fed the adequate diet first (regimen 9 vs. 11).

Tibia Ash and Bone Disease Correlations

Significant negative correlations ($P < 0.05$) were found between 18-d percentage of tibia ash and the incidence of broken tibias and broken femurs in Experiment 1 (Table 6). Significant positive correlations were found between 18-d tibia ash and 35-d tibia ash as well as the incidence of 18-d P rickets and broken tibias and broken femurs. A significant positive correlation was also found between 18-d Ca rickets incidence and the incidence of broken tibias at processing.

The same correlations were found in Experiment 2 (Table 7), with the exception that 18-d percentage of tibia ash was poorly correlated with 35-d percentage of tibia ash and the incidence of broken femurs. Furthermore, 18-d P rickets were not correlated with the incidences of broken femurs, and 18-d Ca rickets were not correlated with broken tibias. The valgus incidence was weakly correlated to the broken tibia incidence.

In Experiment 1, highly significant ($P < 0.001$) negative correlations ($0.5 < |r| < 0.8$) were found between the percentage of tibia ash at 35 d and the incidences of broken tibias, broken clavicles, bloody pectoralis minor muscles, and broken femurs (Table 8). These same correlations were found in Experiment 2, with the exception of the broken femur incidence, for which no significant correlation was found. Furthermore, the correlations obtained in Experiment 2 were weak ($0.3 < |r| < 0.5$) rather than moderate ($0.5 < |r| < 0.8$).

DISCUSSION

Results of the current work suggest that feeding Ca- and P-deficient diets during the ST and GF affects the integrity of the different bones of the chicken in different ways during slaughter and processing. The ability of long bones, such as the tibia and femur, to resist breaking appeared to be influenced by the Ca and P content of the ST diet at a $P < 0.10$ level, whereas the incidence of broken clavicles was influenced only by the type of diet fed during the GF ($P < 0.03$).

The significant correlations between the 18-d percentage of tibia ash and the incidence of broken tibias and broken femurs when birds were slaughtered at 36 d indicates that 18-d tibia ash could be used to predict the integrity of long bones during processing as accurately as 35-d tibia ash values (Tables 6, 7, and 8). Similar correlations found between the 18-d P rickets incidence and the broken tibia incidence indicates that the poor mineralization and lack of bone remodeling associated with P rickets (Long et al., 1984b) early in a commercial broiler's development significantly affected bone strength at 36 d. No such correlations were found for broken clavicles, which suggests that the clavicle, a short bone, is more sensitive to short-term fluctuations in the Ca and P status of the bird, whereas the integrity of the tibia and femur depend more on the Ca and P status of the chick during early life, when bone development is more active.

Table 5. Effect of phytase and 1α-hydroxycholecalciferol (1α-OHD$_3$; P + 1α) supplementation on broken clavicle incidence and breast meat quality of broiler chickens after carcass separation (0 to 35 d), Experiment 2

		Starter (0 to 18 d)			Grower-finisher (19 to 35 d)			Percentage		
Regimen	Repititions	Ca	tP[1]	Supplement[2]	Ca	tP[1]	Supplement[2]	Broken clavicle incidence	Bloody pectoralis minor incidence	Bloody pectoralis major incidence
1	4	0.60	0.47	—	0.30	0.37	—	37.5 ± 6.3^{bc}	47.5 ± 4.3^{abc}	16.3 ± 2.4^{b}
2	4	0.60	0.47	—	0.30	0.37	P	30.0 ± 5.8^{bcd}	48.8 ± 9.0^{abc}	12.5 ± 3.2^{b}
3	4	0.60	0.47	—	0.30	0.37	P + 1α	42.5 ± 7.5^{ab}	51.3 ± 3.8^{abc}	18.8 ± 6.6^{b}
4	4	0.60	0.47	—	0.80	0.67	—	12.5 ± 6.3^{d}	16.3 ± 6.9^{d}	13.8 ± 3.8^{b}
5	4	0.60	0.47	P + 1α	0.30	0.37	—	40.0 ± 12.2^{ab}	57.5 ± 3.2^{ab}	20.0 ± 9.4^{ab}
6	4	0.60	0.47	P + 1α	0.30	0.37	P	46.9 ± 5.1^{ab}	49.1 ± 9.7^{abc}	11.9 ± 5.1^{b}
7	4	0.60	0.47	P + 1α	0.30	0.37	P + 1α	30.0 ± 5.8^{bcd}	33.8 ± 7.7^{bcd}	16.3 ± 2.4^{b}
8	4	0.60	0.47	P + 1α	0.80	0.67	—	15.0 ± 6.5^{cd}	23.8 ± 1.3^{d}	11.3 ± 3.1^{b}
9	4	0.90	0.68	—	0.30	0.37	—	62.5 ± 9.5^{a}	60.0 ± 4.6^{a}	33.8 ± 5.5^{a}
10	4	0.90	0.68	—	0.30	0.37	P	47.5 ± 4.8^{ab}	36.3 ± 12.5^{abcd}	13.8 ± 6.9^{b}
11	4	0.90	0.68	—	0.30	0.37	P + 1α	30.0 ± 10.8^{bcd}	32.2 ± 5.8^{cd}	11.3 ± 1.3^{b}
12	4	0.90	0.68	—	0.80	0.67	—	23.3 ± 5.3^{bcd}	28.8 ± 6.9^{cd}	10.0 ± 3.5^{b}
						ANOVA				
						R^2		0.56	0.61	0.50
						Source	df		*P*-value	
						Regimen	11	0.0021	0.0006	0.0567
						Block	3	0.4577	0.1583	0.0288
						Error	33			

[a–d]Values within variables with no common superscripts differ ($P < 0.05$) when tested with Duncan's multiple range test following ANOVA.

[1]tP = total P.

[2]P + 1α represents 1,000 phytase units (FTU)/kg of Natuphos 5000 (BASF Corp., Wyandotte MI) plus 5 μg/kg of 1α-OHD$_3$. P represents 1,000 FTU/kg of Natuphos 5000.

The high numbers of broken clavicles observed in the current work compared with the other bones may have been because clavicles are small, fine bones that are near the surface of the skin and are therefore vulnerable to damage during stunning (Gregory and Wilkins, 1989, 1990; Kan, 1993; Gregory et al., 1995). In both experiments, broken clavicles were associated with bloody breast meat; however, in many instances, bleeding occurred without any obvious fracturing of the bone. This bleeding may have occurred as the result of the greater propensity of Ca-deficient birds to bleed from hairline bone fractures that were not detectable by visual examination or from a more porous bone due to poor mineralization and remodeling (Rath et al., 2000). Calcium is required for a number of the blood-clotting proteins that may explain this phenomenon (Stenflo, 1991). Gregory et al. (1995) attributed breast hemorrhages without broken clavicles to poor drainage of the blood vessels and the rupture of the capillaries in the muscle during the spasm at stunning.

Tibia ash values, which are usually used as an indicator of bone strength, were better correlated to the incidence of bloody breast muscles, particularly the pectoralis minor muscle, compared with the incidence of fractured tibias, femurs, or clavicles (Table 8). Several studies have been conducted to determine the impact of reducing the inorganic Ca and P content of feed during the finisher phase of broiler production, but most have evaluated carcass quality in terms of broken bones without evaluating the effect of Ca and P on bleeding and bruising of the meat (Skinner et al., 1992a,b; Skinner and Waldroup, 1992; Chen and Moran, 1994). Chen and Moran (1995), however, did determine that omitting dicalcium phosphate from broiler finisher rations caused an increase in blood-splashed breast meat when birds were still alive at the time of bone failure. These results are consistent with the current work and demonstrate that unless sufficient Ca and P are added to the diet to eliminate bloody meat, there is little value in reducing the quantity of the minerals

Table 6. Pearson correlation coefficients (r) for 18-d percentage tibia ash, P rickets, Ca rickets, and tibial dyschondroplasia (TD) incidences against tibia and carcass quality variables of 35 d-old broiler chickens, Experiment 1 (n = 4)

	Tibia ash (18 d)		P rickets incidence (18 d)		Ca rickets incidence (18 d)		TD incidence (18 d)	
Variables	r	*P*-values	r	*P*-values	r	*P*-values	r	*P*-values
P rickets score (18 d)	–0.9436	<0.0001	0.9862	<0.0001	0.0092	0.9660	–0.3746	0.0713
Tibia ash (35 d)	0.6999	0.0001	–0.5701	0.0036	–0.2286	0.2826	0.2441	0.2505
Broken clavicle incidence (35 d)	–0.2066	0.3327	0.1415	0.5096	0.2749	0.1936	0.1276	0.5523
Bloody pectoralis minor incidence (35 d)	–0.3373	0.1070	0.2022	0.3433	0.2154	0.3120	0.1463	0.4951
Bloody pectoralis major incidence (35 d)	–0.2537	0.2316	0.1610	0.4522	0.0883	0.6815	0.1871	0.3814
Broken tibia incidence (35 d)	–0.6568	0.0005	0.6979	0.0001	0.4286	0.0367	–0.2572	0.2250
Broken femur incidence (35 d)	–0.4955	0.0138	0.3491	0.0946	0.2335	0.2721	–0.1441	0.5016

Table 7. Pearson correlation coefficients (r) for 18-d percentage tibia ash, P rickets, Ca rickets, tibial dyschondroplasia (TD), and valgus incidences against tibia and carcass quality variables of 35 d-old broiler chickens, Experiment 2 (n = 4)

	Tibia ash (18 d)		P rickets incidence (18 d)		Ca rickets incidence (18 d)		TD incidence (18 d)		Valgus incidence (18 d)	
Variables	r	*P*-values	r	*P*-values	r	*P*-values	r	*P*-values	r	*P*-values
P rickets score (18 d)	–0.7824	<0.0001	0.9874	<0.0001	–0.1781	0.2259	0.1536	0.2972	0.6250	<0.0001
Tibia ash (35 d)	0.1897	0.2289	–0.2975	0.0557	0.2795	0.0730	–0.0440	0.7819	–0.1156	0.4662
Broken clavicle incidence (35 d)	0.2089	0.1541	–0.1568	0.2871	0.0318	0.8302	–0.0100	0.499	–0.0851	0.5652
Bloody pectoralis minor incidence (35 d)	–0.1435	0.3306	0.0792	0.5928	–0.1943	0.1857	–0.0060	0.9679	0.0597	0.6868
Bloody pectoralis major incidence (35 d)	0.0598	0.6864	–0.0765	0.6055	0.0273	0.8538	0.0285	0.8477	0.0054	0.9708
Broken tibia incidence (35 d)	–0.4456	0.0015	0.2688	0.0647	–0.1093	0.4597	0.1686	0.2519	0.2547	0.0806
Broken femur incidence (35 d)	–0.2760	0.0576	–0.0313	0.8329	–0.0688	0.6420	0.2854	0.0492	0.2959	0.8418

in the diet, even if performance is maximized and broken bones are prevented.

The addition of phytase and 1α-OHD_3 to the deficient diets produced variable effects on the different bones in the 2 experiments. Feeding the supplements in Experiment 1 increased the percentage of tibia ash (Driver et al., 2005) and reduced the incidence of fractures in the long bones, probably due to the ability of both supplements to increase Ca and P retentions (Edwards, 1993; Biehl et al., 1995; Mitchell and Edwards, 1996; Biehl and Baker, 1997). The poor response in the incidence of long-bone fractures to the supplements in Experiment 2 could not easily be explained. The broilers were fed identically formulated rations but were unable to demonstrate the same levels of improvement in performance or tibia ash as birds in the first experiment (Driver et al., 2005). In both experiments, adding the supplements did not significantly ($P > 0.05$) reduce the incidence of broken clavicles or bloody breast meat. These characteristics may be more sensitive to the short-term fluctuations in Ca or P and are, therefore, more susceptible to the very low levels of Ca and P fed in the GF diet. This is also suggested by the fact that birds fed the deficient diet with supplements were unable to attain the same level of tibia ash at 35 d as birds fed the adequate diet in either experiment (Driver et al., 2005).

In conclusion, in 2 experiments that closely approximated commercial growout and processing conditions, the incidence of broken tibias and femurs during evisceration and carcass dissection were influenced by the Ca and P content of diets fed during both the first 18 and last 19 to 35 d of age. The Ca and P content of the diets fed during the first 18 d had little effect on the incidence of broken clavicles and the incidence of bloody breast meat. These characteristics appeared to be influenced by the short-term Ca and P status of the birds rather than the Ca and P status during bone development. The addition of phytase and 1α-OHD_3 to increase dietary Ca and P retention reduced the incidence of broken tibias and femurs but did less to reduce the incidence of broken clavicles or bloody breast meat. Bloody breast meat was sometimes observed without concurrent clavicle fractures.

Low levels of Ca and P were chosen for both experiments so that the possibility of restoring normal growth and performance by adding phytase and 1α-OHD_3 to the diet could be investigated. Higher levels of Ca and P are necessary to return all parameters, including bloody breast meat, to adequate control levels.

Experiment-to-experiment variation remains a concern in experiments of this nature. Broilers responded differently to the same diet formulations in different, apparently identical experiments. Further investigation of factors responsible for the variability obtained in such experiments needs to be conducted.

Table 8. Pearson correlation coefficients (r) between percentage tibia ash and carcass quality variables of 35 d-old broiler chickens evaluated after processing, Experiment 1 and Experiment 2 (n = 4)

	Experiment 1		Experiment 2	
Variables	r	*P*-values	r	*P*-values
Broken tibia incidence	–0.4078	0.0479	–0.3107	0.0452
Broken femur incidence	–0.5316	0.0075	–0.1490	0.3464
Broken clavicle incidence	–0.5324	0.0074	–0.3079	0.0473
Bloody pectoralis minor incidence	–0.6357	0.0009	–0.5558	0.0001
Bloody pectoralis major incidence	–0.3601	0.0839	–0.2323	0.1388

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