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Effect of dietary calcium concentrations in low non-phytate phosphorus diets containing phytase on growth performance, bone mineralization, litter quality, and footpad dermatitis incidence in growing broiler chickens

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Objective: An experiment was conducted to investigate the effect of dietary Ca concentrations in low non-phytate phosphorus (NPP) diets containing phytase on growth performance, bone mineralization, litter quality, and footpad dermatitis (FPD) incidence in growing broiler chickens.
Methods: A total of 1,800 21-day-old Ross 308 growing broiler chickens were allotted to 1 of 6 dietary treatments with 6 replicated cages. Six diets were formulated to provide increasing Ca concentrations of 4.0, 5.0, 6.0, 7.0, 8.0, or 9.0 g/kg in diets. The concentrations of NPP in all diets were maintained at 3.0 g/kg, and phytase was supplemented to all diets at the level of 1,000 fytase units (FTU)/kg. At the end of the 14-d feeding trial, birds were euthanized for tibia sampling, and litter samples were collected from 3 areas in the cage. The FPD incidence was measured based on a 6-point scoring system.
Results: Dietary Ca concentrations had no effect on growth performance of growing broiler chickens. However, a tendency (linear, $p = 0.05$) for decreased feed efficiency was observed as dietary Ca concentrations were increased. The concentrations of Ca and P in the tibia of broiler chickens increased (linear and quadratic, $p<0.01$) with increasing Ca concentrations in low NPP diets containing phytase. Litter pH, moisture, and N contents were not affected by increasing Ca concentrations in low NPP diets containing phytase. However, a tendency (quadratic, $p = 0.10$) for increased FPD incidence with increasing dietary Ca concentrations was observed.
Conclusion: Dietary Ca concentrations from 4.0 to 9.0 g/kg in low NPP diets containing phytase have little effects on growth performance of growing broiler chickens. However, Ca and P concentrations in the tibia are decreased if dietary Ca concentrations are less than 5.0 g/kg. The FPD incidence for growing broiler chickens may be decreased if less than 9.0 g/kg of Ca is included in diets.

Keywords: Broiler Chicken; Dietary Calcium Concentration; Footpad Dermatitis; Growth Performance; Litter Quality; Phytase

INTRODUCTION

Calcium (Ca) and phosphorus (P) are the most important minerals in poultry diets to obtain the optimal productivity. Currently, the National Research Council (NRC) has recommended 9.0 g/kg Ca and 3.5 g/kg non-phytate phosphorus (NPP) for growing broiler chickens [1]. However, the recommended dietary concentrations of Ca and P for broiler chickens are still debatable. In addition, there are increasing environmental and economic concerns regarding P utilization, and therefore, broiler nutritionists currently make a great effort to minimize P concentrations in diets to the levels that do not damage broiler productivity. Thus, the current broiler industry has developed and adopted low NPP diets. Moreover, phytase is also widely used to optimize P utilization in the low NPP diets to date [2].

Dietary Ca concentrations are suggested to be adjusted according to dietary NPP concentrations due to potential antagonising effects of Ca on P utilization in the gastrointestinal tract of poultry [3]. This adjustment is likely more important when low NPP diets are fed to broiler chickens [4,5]. Furthermore, previous experiments have reported that Ca utilization can be improved by supplemental phytase in addition to improved P utilization [2,6]. Therefore, a greater reduction in the concentrations of Ca in low NPP diets containing phytase than the current NRC recommendations [1] can be practiced for broiler diets. However, limited data regarding the relationship between Ca and NPP concentrations in diets containing phytase are available for broiler chickens. In addition, Collett [7] suggested that high concentrations of Ca in poultry diets may impair litter quality by increasing litter moisture content. In addition, poor litter quality is often associated with footpad dermatitis (FPD) incidence, which has gained increasing attention in broiler industry [8]. Thus, we hypothesised that the FPD incidence would be decreased when broiler chickens are fed diets containing low concentrations of Ca. However, no experiments using growing broiler chickens have been conducted, in which dietary Ca concentration and FPD incidence has been studied.

The objective of the present experiment, therefore, was to investigate the effect of dietary Ca concentrations in low NPP diets containing phytase on growth performance, bone mineralization, litter quality, and FPD incidence in growing broiler chickens.

MATERIALS AND METHODS

Birds, diets, and experimental design

The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at Chung-Ang University.

A total of 1,800 21-day-old Ross 308 growing broiler chickens (initial body weight [BW] = 962±57.0 g) were used and raised in conventional floor pens (200×230×100 cm, width×length×height) for 14 days of growing period. Before the start of the experiment, all chickens were fed a commercial starter diet. All chickens were allotted to 1 of 6 dietary treatments with 6 replicates in a completely randomized design. Each replicate had 50 birds per cage. Six commercial-type experimental diets were formulated to provide different Ca concentrations of 4.0, 5.0, 6.0, 7.0, 8.0, or 9.0 g/kg in diets (Table 1). The concentrations of NPP in all diets were maintained at 3.0 g/kg, which was less by 15% than the current recommendation of NPP concentrations (3.5 g/kg) in diets fed to growing broiler chickens [1]. Phytase (Phyzyme XP, Danisco Animal Nutrition, Marlborough, UK) was also supplemented to all diets at the level of 1,000 fytase units/kg as simulated to the commercial type phytase-containing diets. The experimental diets were mash form. All diets were formulated to meet or exceed the National Research Council requirements for growing broiler chickens [1], with the exception of Ca and NPP (Table 1). The diets and water were provided *ad libitum* throughout the experiment. Initial room temperature was set at 24°C, and was maintained from 23°C to 25°C during the experiment. Fresh rice hulls were used as bedding materials in the cages at the start of the experiment. Each cage had 2 separate feeders and 1 automatic water bowl. A 24-hour lighting schedule was used throughout the experiment. The body weight gain (BWG) and feed intake (FI) were recorded at the end of the experiment. Mortality was recorded daily. Feed efficiency (G:F) was calculated by dividing BWG with FI that were adjusted with mortality.

Sample collection and chemical analysis

At the conclusion of the experiment (35 days of age), 2 birds per replicate with a BW close to the replicate mean BW (i.e., 12 birds per treatment) were euthanized by CO_2 asphyxiation, then immediately dissected. Both left and right tibias were collected from each replicate (i.e., 2 birds) before analysis. The right tibias were detached from adhering tissues, and then dried at 100°C for 24 hours in a drying oven. Fat in dried right tibias was extracted with ethyl ether in a Soxhlet apparatus for 48 hours. Afterwards, the samples were dried at 100°C for 24 hours in a drying oven, then dried right tibias were ground to pass through a 1.0-mm screen. Grounded samples were ashed for 24 hours at 600°C in a muffle furnace to measure tibia ash contents [9,10]. Tibia Ca and P concentrations were analyzed in the ash of right tibia by inductively coupled plasma spectrometer (Optima 5300 DV, Perkin Elmer Inc., Shelton, CT, USA) as proposed by Kurtoğulu et al [11] with minor modification. The left tibias were used for breaking strength analysis using a texture analyser TA-HDi (Stable Micro Systems Ltd, Surrey, UK) according to the method described by Shaw et al [12].

Litter samples were collected from 3 areas in the cage (i.e., near the waterer, near the feeder, and at the center) at the end of the experiment. The collected litter samples were stored in the refrigerator at –20°C until further analysis. Litter pH was determined by the method described by Pope and Cherry [13]. Litter moisture concentrations were measured by drying oven at 100°C for 12 hours. Litter nitrogen (N) concentrations were determined using the Kjehldahl method [14]. At the conclusion of the experiment, the FPD incidence was also measured visually using 10 birds randomly selected per pen based on a 6-point scale scoring system [15]: score 0, smooth and no lesion; score 1, discoloration and small injury; score 2, dark papillae and no ulceration; score 3, small ulcer covered by crust; score 4, medium ulcer covered by crust; and score 5, big ulcer covered by crust.

Statistical analysis

All data were tested for normal distribution and outliers with the UNIVARIATE procedure of SAS (SAS Institute Inc., Cary,

Table 1. Composition and nutrient content of experimental diets

Items (g/kg, unless noted)	Dietary treatments (Calcium concentrations of diets, g/kg)[1)]					
	4.0	5.0	6.0	7.0	8.0	9.0
Ingredients						
Corn	501.5	501.8	502.0	502.0	502.0	501.8
Soybean meal (46% CP)	151.4	149.5	148.6	151.6	153.2	154.8
Wheat	100.0	100.0	117.7	123.5	117.4	111.4
Wheat flour	89.2	85.0	62.8	50.0	50.0	50.0
Corn gluten meal	40.4	45.0	45.0	45.0	45.0	45.0
Poultry by-product	40.0	37.4	40.0	40.0	40.0	40.0
Rapeseed meal	20.0	20.0	20.0	20.0	20.0	20.0
Full fat soybean	10.0	10.0	10.0	10.0	10.0	10.0
Soybean oil	10.0	10.0	10.0	10.0	10.0	10.0
Animal fat	9.2	10.0	10.0	11.4	13.2	15.2
Salt	2.5	2.5	2.5	2.5	2.5	2.5
Mono-dicalcium phosphate	8.6	8.6	8.6	8.6	8.6	8.6
Limestone	2.6	5.4	8.0	10.6	13.4	16.0
Sodium bicarbonate	1.6	1.6	1.6	1.6	1.6	1.6
Threonine	0.7	0.7	0.7	0.7	0.7	0.7
DL-methionine	3.7	3.7	3.7	3.7	3.7	3.7
L-lysine sulfate	5.0	5.2	5.2	5.2	5.1	5.1
Choline-Cl	0.6	0.6	0.6	0.6	0.6	0.6
Vitamin premix[2)]	1.4	1.4	1.4	1.4	1.4	1.4
Mineral premix[3)]	1.4	1.4	1.4	1.4	1.4	1.4
Phytase[4)]	0.2	0.2	0.2	0.2	0.2	0.2
Calculated nutrient content[5)]						
AMEn (MJ/kg)	12.9	12.9	12.9	12.9	12.9	12.9
Crude protein	195.0	195.0	195.0	195.0	195.0	195.0
Total lysine	11.0	11.0	11.0	11.0	11.0	11.0
Total methionine+cysteine	9.0	9.0	9.0	9.0	9.0	9.0
Total threonine	7.5	7.5	7.5	7.5	7.5	7.5
Total tryptophan	2.0	2.0	2.0	2.0	2.0	2.0
Calcium	4.0	5.0	6.0	7.0	8.0	9.0
Non-phytate phosphorus	3.0	3.0	3.0	3.0	3.0	3.0
Analyzed nutrient content						
Calcium	5.2	6.6	7.4	8.6	9.2	9.7
Total phosphorus	5.3	5.3	5.3	5.4	5.5	5.0

CP, crude protein; AME_n, nitrogen-corrected apparent metabolizable energy.
1) Dietary treatments = formulated calcium concentrations of diets.
2) Provided per kg of the complete diet: vitamin A, 12,600 IU (retinyl acetate); vitamin D_3, 3,080 IU; vitamin E, 42 IU (DL-alpha-tocopheryl acetate); vitamin K_3, 2.8 mg (menadione dimethpyrimidinol); vitamin B_1, 2.8 mg; vitamin B_2, 7.28 mg; vitamin B_6, 4.2 mg; vitamin B_{12}, 28 μg; folic acid, 1.4 mg; biotin, 130 μg; niacin, 42 mg.
3) Provided per kg of the complete diet: iron, 60 mg ($FeSO_4$); zinc, 90 mg ($ZnSO_4$); manganese, 97.5 mg (MnO); copper, 6.75 mg ($CuSO_4$); cobalt, 530 μg ($CoSO_4$); selenium, 200 μg (Na_2SeO_3); iodine, 2 mg ($Ca[IO_3]_2$).
4) Phyzyme XP (Danisco Animal Nutrition, Marlborough, UK).
5) Calculated values [1].

NC, USA). No outliers were identified. All data except for FPD incidence were normally distributed. Thus, data with normal distribution were analyzed by the MIXED procedure of SAS, whereas data for FPD incidence were analyzed by the GLIMMIX procedure of SAS [16]. The LSMEANS procedure was used to calculate mean values. The orthogonal polynomial contrast test was also performed to determine linear and quadratic effects of increasing Ca concentrations in diets. Significance and tendency for statistical tests were set at $p<0.05$ and $0.05\leq p\leq 0.10$, respectively.

RESULTS

Growth performance including final BW, BWG, FI, G:F, and mortality was not affected by dietary treatments (Table 2). A tendency (linear, $p = 0.05$) for decreased G:F was observed as dietary Ca concentrations were increased.

As expected, increasing Ca concentrations in low NPP diets containing phytase increased (linear and quadratic, $p<0.01$) the concentrations of Ca and P in the tibia of growing broiler chickens, but had no effects on tibia ash contents and breaking strength

Table 2. Effects of dietary calcium concentrations in low non-phytate phosphorus diets containing phytase on growth performance of growing broiler chickens[1)]

Items	Dietary Ca concentrations (g/kg)						SEM	p-value[2)]		
	4.0	5.0	6.0	7.0	8.0	9.0		T	L	Q
Final body weight (g)	1,931	1,964	1,912	1,954	1,888	1,889	29.1	0.32	0.11	0.42
Body weight gain (g)	971	1,004	951	988	924	928	32.1	0.43	0.13	0.54
Feed intake (g)	1,684	1,691	1,669	1,710	1,660	1,694	27.6	0.82	1.00	0.93
Feed efficiency (g/kg)[3)]	577	593	569	578	556	548	14.8	0.32	0.05	0.42
Mortality (%)	0.3	2.7	1.3	2.7	2.3	2.3	0.88	0.37	0.17	0.35

SEM, standard error of the mean.
1) Data are least squares means of 6 observations per treatment.
2) T, overall effects of treatments; L, linear effects of increasing concentrations of Ca in diets; Q, quadratic effects of increasing concentrations of Ca in diets.
3) Gain to feed ratio.

(Table 3). Notably, broiler chickens fed diets containing 4.0 g/kg Ca concentrations had the least ($p<0.05$) concentrations of Ca and P in the tibia among those fed experimental diets.

Increasing Ca concentrations in low NPP diets containing phytase had no effects on litter quality including pH, moisture, and N contents (Table 4). However, a tendency (quadratic, $p = 0.10$) for increased FPD incidence was observed as dietary Ca concentrations were increased with the highest FPD incidence being observed for birds fed diets containing 9.0 g/kg Ca.

DISCUSSION

The analyzed concentrations of Ca in diets (5.2, 6.6, 7.4, 8.6, 9.2, or 9.7 g/kg) were greater by about 0.7 to 1.9 g/kg compared with the formulated concentrations of Ca in diets (4.0, 5.0, 6.0, 7.0, 8.0, or 9.0 g/kg). Similar differences between analyzed and formulated concentrations of Ca in diets have been reported in the previous experiments [17,18]. This difference is probably associated not only with possible underestimation of Ca concentrations of ingredients used in the diet formulation but also with the use of calcium carbonate carrier in vitamin and mineral premix used in diet formulation [18].

The observation that growth performance including BW, BWG, FI, and mortality was not affected by dietary treatments agrees with Rousseau et al [19] who reported that increasing Ca concentrations from 3.7 to 7.7 g/kg in diets had no effects on growth

Table 3. Effects of dietary calcium concentrations in low non-phytate phosphorus diets containing phytase on tibia characteristics of growing broiler chickens[1)]

Items	Dietary Ca concentrations (g/kg)						SEM	p-value[2)]		
	4.0	5.0	6.0	7.0	8.0	9.0		T	L	Q
Tibia ash (%)	52.1	52.0	52.7	51.4	52.5	53.3	0.47	0.13	0.13	0.18
Tibia Ca (%)	42.5[c]	46.4[b]	49.7[a]	47.6[ab]	48.4[ab]	48.4[ab]	1.08	< 0.01	< 0.01	< 0.01
Tibia phosphorus (%)	26.0[c]	27.8[b]	28.8[ab]	28.8[ab]	29.2[a]	29.0[ab]	0.44	< 0.01	< 0.01	< 0.01
Tibia breaking strength (kg/cm^2)	20.4	19.5	22.3	20.1	23.7	22.0	1.87	0.60	0.24	0.97

SEM, standard error of the mean.
1) Data are least squares means of 6 observations per treatment.
2) T, overall effects of treatments; L, linear effects of increasing concentrations of Ca in diets; Q, quadratic effects of increasing concentrations of Ca in diets.
a-c Means with different superscripts within a row are different ($p < 0.05$).

Table 4. Effects of dietary calcium concentrations in low non-phytate phosphorus diets containing phytase on litter quality and footpad dermatitis (FPD) incidence of growing broiler chickens[1)]

Items	Dietary Ca concentrations (g/kg)						SEM	p-value[2)]		
	4.0	5.0	6.0	7.0	8.0	9.0		T	L	Q
Litter pH	7.97	7.88	7.95	7.91	8.00	7.91	0.082	0.91	0.99	0.88
Litter moisture (%)	42.33	44.05	42.37	39.15	37.01	45.65	3.106	0.41	0.77	0.26
Litter nitrogen (%)	1.89	2.05	1.94	2.01	1.97	1.83	0.139	0.90	0.68	0.36
FPD incidence [3)]	1.58	1.70	1.62	1.22	1.52	2.37	0.339	0.31	0.30	0.10

SEM, standard error of the mean.
1) Data are least squares means of 6 observations per treatment.
2) T, overall effects of treatments; L, linear effects of increasing concentrations of Ca in diets; Q, quadratic effects of increasing concentrations of Ca in diets.
3) The FPD incidence was measured visually using 10 birds randomly selected per pen (i.e., replicate) based on a 6-point scale scoring system: score 0, smooth and no lesion; score 1, discoloration and small injury; score 2, dark papillae and no ulceration; score 3, small ulcer covered by crust; score 4, medium ulcer covered by crust; and score 5, big ulcer covered by crust [15].

performance of growing broiler chickens. Our result indicates that the concentrations of Ca from 4.0 to 9.0 g/kg in low NPP diets containing phytase did not significantly limit growth performance of growing broiler chickens. However, increasing Ca concentrations in diets tended to decrease the G:F. Powell et al [20] also reported a similar decrease in the G:F of broiler chickens fed diets containing high Ca concentrations. However, Rousseau et al [19] showed no significant difference in the G:F of growing broiler chickens fed diets containing 3.7, 5.7, or 7.7 g/kg Ca concentrations. The reason for these variable results among experiments may be associated with the difference in the Ca:NPP ratio in experimental diets. The current NRC recommendation for the Ca:NPP ratio is 2.6:1 (9.0 g/kg Ca:3.5 g/kg NPP) at 3 to 6 weeks of age [1]. However, this ratio has been questionable because of changes in genetic development, management, and raising environment of modern broiler industry. Currently, low NPP diets containing phytase, which is similar to our experimental diets, have been widely used in the broiler industry. In this situation, the optimal ratio for broiler chickens should be adjusted [21]. In the present experiment, the reduction up to 1.3:1 (4.0 g/kg Ca:3.0 g/kg NPP) of Ca:P in low NPP diets containing phytase had no detrimental effects on growth performance of growing broiler chickens.

In contrast to growth performance, however, significantly low Ca and P concentrations in the tibia were observed for growing broiler chickens fed diets containing 4.0 g/kg Ca although there were no differences in tibia ash and breaking strength among treatments. This result indicates that the reduction up to 1.7:1 (5.0 g/kg Ca:3.0 g/kg NPP) of Ca:P in low NPP diets containing phytase may have little negative effects on bone mineralization of growing broiler chickens. However, one should be cautious in considering the current data because the feeding duration of experimental diets in this experiment was only 14 days, which may be too short to impair bone mineralization of growing broiler chickens.

It is suggested that wet litter problems may be exacerbated by high concentrations of Ca in broiler diets because poultry have a limited capacity of reabsorbing Ca in the kidney, leading to a polyuria [7,22]. In the current experiment, however, birds fed low NPP diets containing increasing Ca concentrations from 4.0 to 9.0 g/kg had no effects on litter moisture contents. This result is probably due to the fact that increasing Ca concentrations from 4.0 to 9.0 g/kg in low NPP diets containing phytase did not interrupt renal reabsorption capacity of Ca. In the line with litter moisture content, litter pH and N contents were not affected by increasing Ca concentrations in diets. Therefore, it can be elucidated that dietary Ca concentrations in the range of 4.0 to 9.0 g/kg may have no negative effects on litter pH and N contents.

The FPD has been often related to poor litter quality such as high litter moisture and N contents [8]. However, we observed the highest FPD incidence in growing broiler chickens fed diets containing 9.0 g/kg Ca, which is the current Ca recommendation of NRC [1], despite no differences in litter quality among dietary treatments. The reason for little association between litter quality and FPD incidence as observed in this experiment is not clear; it may be related to the other factors affecting litter quality such as animal conditions, temperature, humidity, bedding materials, and litter microbial population, possibly in relation to different Ca concentrations in diets [8]. However, our result indicates that FPD incidence for growing broiler chickens may be decreased if less than 9.0 g/kg of Ca was included in diets.

CONCLUSION

Dietary Ca concentrations from 4.0 to 9.0 g/kg in low NPP diets containing phytase have little effects on growth performance of growing broiler chickens during 14 days of feeding. However, Ca and P concentrations in the tibia are decreased if dietary Ca concentrations are less than 5.0 g/kg. The FPD incidence for growing broiler chickens may be decreased if less than 9.0 g/kg of Ca is included in diets.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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