Effect of calcium level and phytase addition on ileal phytate degradation and amino acid digestibility of broilers fed corn-based diets[1]

A. M. Amerah,*[2] P. W. Plumstead,* L. P. Barnard,* and A. Kumar†

*Danisco Animal Nutrition, DuPont Industrial Bioscience, Marlborough, SN8 1XN, UK; and †School of Agriculture and Food Science, University of Queensland, Gatton, Qld-4343, Australia

ABSTRACT This study investigated the effect of dietary Ca to available P (AvP) ratio and phytase supplementation on bone ash, ileal phytate degradation, and nutrient digestibility in broilers fed corn-based diets. The experimental design was a 4 × 2 factorial arrangement of treatments evaluating 4 Ca:AvP ratios (1.43, 2.14, 2.86, and 3.57) and 2 levels of phytase (0 and 1,000 phytase units/kg of feed). The 4 Ca:AvP ratios were achieved by formulating all diets to a constant AvP level of 0.28% and varying Ca levels (0.4, 0.6, 0.8, and 1.0%). Each treatment was fed to 6 cages of 8 male Ross 308 broilers from 5 to 21 d. At 21 d, digesta from the terminal ileum was collected and analyzed for energy, phytate, P, Ca, and amino acids (AA) to determine digestibility. Digesta pH was measured in each segment (crop, gizzard, duodenum, and ileum) of the digestive tract. Data were analyzed by 2-way analysis of covariance. There was a significant interaction between dietary Ca:AvP ratio and phytase supplementation for weight gain (WG), feed intake (FI), and feed conversion ratio (FCR). In diets with no phytase, Ca:AvP ratio had a greater effect on WG, FI, and FCR compared with those fed diets without phytase. The orthogonal polynomial contrasts showed that the increase in dietary Ca:AvP ratio significantly decreased WG and FI in a quadratic manner, whereas FCR increased ($P < 0.05$) linearly with higher dietary Ca:AvP ratio. Increasing dietary Ca:AvP ratio led to a significant quadratic decrease in phytate degradation and significant linear decreases in P digestibility and bone ash. Phytase addition increased ($P < 0.05$) phytate degradation and improved ($P < 0.05$) energy, AA, and P digestibility at all levels of Ca:AvP with no interaction ($P > 0.05$) between the main factors. Digestibility of AA was positively correlated ($P < 0.05$) with the degree of phytate degradation. Increasing dietary Ca:AvP ratio significantly increased gizzard pH in a linear manner. In conclusion, phytase (1,000 phytase units/kg of feed) improved phytate, and P and AA digestibility at all Ca:AvP ratios evaluated in this study.

Key words: broiler, available phosphorus, calcium, amino acid, phytate degradation

2014 Poultry Science 93:906–915
http://dx.doi.org/10.3382/ps.2013-03465

INTRODUCTION

Microbial phytases have been widely used in poultry feeds as a means of improving dietary P availability and reducing P excretion in manure. The effect of microbial phytases on phytate degradation and subsequent improvements in P utilization in poultry diets has been well documented (Selle and Ravindran, 2007). Several studies have also shown that dietary additions of microbial phytase to poultry diets enhanced the utilization of nutrients other than P, including energy and amino acids (**AA**; Ravindran et al., 1999; Selle et al., 2000; Selle and Ravindran, 2007). However, the magnitude of response has not been consistent in all studies, and factors contributing to the variability in energy and AA digestibility improvements in response to supplemental phytase have not been delineated (Selle and Ravindran, 2007). Potential factors that have been reported to contribute to the variation in response have included the concentration of the substrate for the enzyme (phytate) in the diet, the level of added phytase, the intrinsic properties of the phytase enzyme used, source of phytase, and feed particle size (Ravindran et al., 2006; Amerah and Ravindran, 2009; Selle et al., 2009). Another factor that may have contributed to the inconsistent results in the literature was the formation of Ca-phytate complexes that were not susceptible to degradation by phytase (Angel et al., 2002; Selle et al., 2009).

Received July 4, 2013.
Accepted November 29, 2013.

[2]Corresponding author: Ahmed.amerah@dupont.com

The ability of phytase enzymes to hydrolyze phytate has been shown to be negatively affected by high levels of dietary Ca or a high ratio of Ca:AvP (Qian et al., 1997). As Ca-phytate complexes were formed at pH ≥5 (Selle et al., 2009), similar to the conditions in the small intestine, rapid phytate degradation in the proximal gut where the digesta was more acidic (gizzard + proventriculus) using phytase enzymes has been proposed to mitigate the negative effect of Ca on phytate degradation and increase nutrient availability for the bird. However, phytases have been suggested to vary in their pH optima with efficiency and speed of hydrolyzing phytate depending on their source (Tran et al., 2011). Therefore, the objectives of this study were to examine the interactions of dietary Ca:AvP ratio and addition of a bacterial phytase isolated from *Buttiauxella* spp. on ileal phytate degradation, Ca, P, and AA digestibility in broiler chickens.

MATERIALS AND METHODS

Birds and Housing

Experimental procedures were conducted in accordance with the University of Queensland Animal Ethics Committee guidelines and model code of practice for welfare of animals. A total of 384 one-day-old male Ross 308 broiler chickens were obtained from a commercial hatchery. One-day-old chickens were selected according to mean BW and 8 chickens of 42 ± 3 g were allocated to each of 48 digestibility cages housed in an environmentally controlled room. Feed and water for ad libitum consumption was offered during the trial period. Brooding temperature was maintained at 31°C for the first 7 d then gradually reduced to 27°C at the end of the 3-wk experimental period. The broiler chickens in this trial were on 23 h light with a minimum light intensity of 20 lx and 1 h dark per day. The lighting program and the environment in the house were controlled by timers and temperature sensors, respectively. All chickens were vaccinated against infectious bronchitis at the hatchery. Body weight and feed intake (**FI**) were recorded by cage at 5 and 21 d of age. Mortality was recorded daily. Any bird that died was weighed and feed conversion ratio (**FCR**) values were calculated by dividing total feed intake by weight gain (**WG**) of live plus dead birds.

Diets and Treatments

From 1 to 5 d, all birds in the experiment received a commercial broiler starter diet. The experimental diets were offered from 5 to 21 d of age and were based mainly on corn and soybean meal (Table 1). Diets were formulated to be similar to commercial diets fed to the Ross 308 strain of broiler, except Ca and P. This study had 8 experimental diets. Four basal diets were formulated with different Ca:AvP ratios (1.43, 2.14, 2.86, and 3.57), and each basal diet was then divided into 2 equal parts. One part was supplemented with phytase, and the other part remained unsupplemented. The phytase (Axtra PHY, Danisco Animal Nutrition, Marlborough, UK) was added to provide 1,000 phytase units (**FTU**)/kg of feed. Celite, a source of acid-insoluble ash (**AIA**), was added at 0.2% to all experimental diets as an indigestible marker to determine nutrient digestibility.

Measurements

At 21 d of age, all birds in each group were euthanized by cervical dislocation. The ileum was then immediately excised and divided into 2 parts, the anterior and posterior ileum. The ileum was defined as the portion of the small intestine extending from Meckel's diverticulum to a point 40 mm proximal to the ileocecal junction. Contents of the posterior ileum were collected by gently flushing with distilled water into plastic containers. Digesta were pooled within a cage, lyophilized, ground to pass through a 0.5-mm screen size, and stored at −20°C until analyzed for gross energy, nitrogen, Ca, P, phytate, and AA. At 21 d of age, pH of the contents of the crop, gizzard, duodenum, and ileum was also measured using an Alpha pH test meter (Coral Cay Health, Surfers Paradise, Queensland, Australia) and the left tibia from each bird was collected for bone ash analysis.

Chemical Analysis

Gross energy was determined from the temperature increase when the sample was ignited in an oxygen-rich atmosphere in a bomb calorimeter (Leco AC600, NSW, Australia). Nitrogen was determined by the AOAC method 990.03 (AOAC, 1990) where the sample was ignited in an oxygen atmosphere and the nitrogen determined in the gas stream by a thermal conductivity meter after stripping out the oxygen and replacing it with helium. Calcium and total P in both feed and ileal samples were determined by ICP-OES method 2011.14 (AOAC, 1990) analysis following microwave-assisted acid digestion. The AA analysis was performed using 6 *M* HCl liquid hydrolysis with AA quantitation by AccQTag derivatization (Waters Corporation, Milford, MA) and reversed-phase ultra-performance liquid chromatography analysis (Cohen and DeAntonis, 1994). Left tibia bones were autoclaved for 30 min, and the adhering tissue was removed mechanically. The clean bones were dried in an oven at 100°C for 24 h, and dried bones were ground. According to AOAC method 942.05 (AOAC, 1990), 2 g of ground bone sample was ashed at 600°C using a thermo gravimetric analyzer. Calcium and P contents of tibia ash were analyzed by the ICP-OES method 2011.14 (AOAC, 1990). Phytate in both feed and ileal samples was extracted by the method described by Latta and Eskin (1980) and determined by ICP-AES. Acid insoluble ash was determined by

Table 1. Composition and calculated analysis (g/100 g as fed) of the basal diet[1]

	Formulated Ca:available P ratio			
Item	1.43	2.14	2.86	3.57
Ingredient				
Corn	62.36	61.33	60.29	59.25
Soybean meal 48% CP	34.03	34.21	34.40	34.58
Soybean oil	0.68	1.01	1.34	1.66
DL-Methionine	0.31	0.31	0.31	0.31
L-Threonine	0.12	0.12	0.12	0.12
L-Lysine HCl	0.30	0.30	0.29	0.29
Salt	0.32	0.33	0.33	0.33
Limestone	0.30	0.83	1.35	1.87
Dicalcium phosphate	0.98	0.98	0.98	0.98
Vitamin and trace mineral premix[1]	0.30	0.30	0.30	0.30
Indigestible marker	0.20	0.20	0.20	0.20
Calculated analysis				
CP	22.00	22.00	22.00	22.00
ME, kcal/kg	2,990	2,990	2,990	2,990
Total P	0.55	0.55	0.55	0.55
Available P	0.28	0.28	0.28	0.28
Ca	0.40	0.60	0.80	1.00
Digestible lysine	1.24	1.24	1.24	1.24
Digestible methionine + cysteine	0.87	0.87	0.87	0.87
Analyzed value				
Ca	0.51	0.68	0.91	1.30
Total P	0.51	0.51	0.51	0.51
Phytate P	0.32	0.32	0.32	0.32

[1]Supplied per kilogram of diet: antioxidant, 50 mg; biotin, 0.2 mg; calcium pantothenate, 12 mg; cholecalciferol, 60 μg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 50 mg; pyridoxine, 10 mg; *trans*-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; DL-α-tocopheryl acetate, 40 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 μg; Zn, 60 mg.

AOAC method 975.12 (AOAC, 1990) by boiling ash in 25 mL of HCl for 5 min, collecting insoluble matter on an ashless filter, washing with hot water until washings are acid-free, igniting until chloride-free, cooling, and weighing the sample.

Calculations

The apparent ileal nutrient digestibility/absorption coefficients were calculated by the following formula using AIA as the indigestible marker (Ravindran et al., 2006):

$$\text{apparent nutrient digestibility } \% = \{[(\text{NT/AIA})\text{d} - (\text{NT/AIA})\text{i}]/(\text{NT/AIA})\text{d}\} \times 100,$$

where (NT/AIA)d = ratio of nutrient and AIA diet, and (NT/AIA)i = ratio of nutrient and AIA in ileal digesta.

Data Analysis

Data were analyzed by 2-way analysis of covariance using the GLM procedure of SAS Institute Inc. (2004, Cary, NC) using cage mean as an experimental unit. A probability value of $P < 0.05$ was described to be statistically significant. Orthogonal polynomial contrasts were used to assess the significance of linear or quadratic models to describe the response in the dependent variable to Ca:AvP level with or without added phytase using the PROC MIXED procedure of SAS.

RESULTS

Diet Analysis

Analyzed dietary Ca, P, and phytate P are shown in Table 1. The analyzed dietary phytate P level was higher than calculated values, which can be explained by higher phytate P values than expected in the feed ingredients used. The analyzed phytate P values for corn and soybean meal were 0.27 and 0.61%, respectively. The calculated values for phytate P for corn and soybean meal were 0.18 and 0.42%, respectively. Phytase recovery was below target but within an acceptable range (mean 831 FTU/kg of diet). Analyzed Ca was also slightly higher and analyzed P slightly lower than formulated values and would have increased the ratio of Ca:AvP slightly above formulated values.

Bird Performance

Effects of dietary Ca:AvP ratio and phytase supplementation on WG (Figure 1), FI, and FCR are summarized in Table 2. There was a significant interaction between dietary Ca:AvP ratio and phytase supplementation for WG, FI, and FCR. In diets with no phytase, Ca:AvP ratio had a greater effect on WG, FI, and FCR compared with those fed diets without phytase. The orthogonal polynomial contrasts showed that the increase in dietary Ca:AvP ratio significantly decreased WG and FI in a quadratic manner, whereas FCR increased ($P < 0.05$) linearly with higher dietary Ca:AvP ratio.

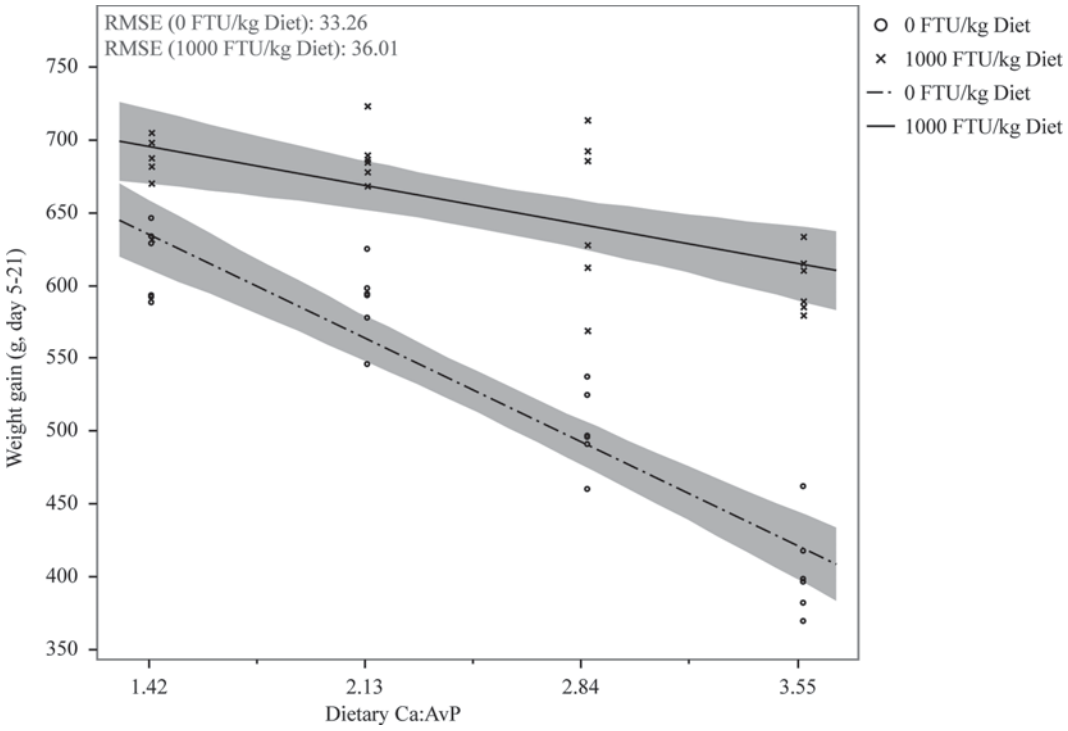

Figure 1. Regression of weight gain in broilers fed diets containing different Ca:available P (AvP) ratios, in the presence and absence of a supplementary microbial phytase from *Buttiauxella*. RMSE = root mean square error; FTU = phytase units.

Table 2. Effect of varying dietary Ca:available P (AvP) ratios and microbial phytase on the weight gain (g/bird), feed intake (g/bird), and feed conversion ratio (g/g) in broilers fed a corn/soy-based diet (5–21 d posthatch)[1]

Item	Phytase (FTU/kg)	Weight gain	Feed intake	Feed conversion ratio
Ca:AvP ratio				
1.43	0	614	819	1.337
2.14	0	589	791	1.345
2.86	0	500	687	1.375
3.57	0	404	596	1.482
1.43	1,000	679	862	1.269
2.14	1,000	688	877	1.274
2.86	1,000	650	854	1.316
3.57	1,000	607	745	1.234
SEM[2]		9.0	24	0.04
Main effect				
Ca:AvP ratio				
1.43		646	841	1.303
2.14		639	834	1.309
2.86		575	770	1.345
3.57		496	670	1.358
Phytase (FTU/kg)				
0		527	723	1.384
1,000		658	834	1.273
$P \leq$				
Ca:AvP		<0.0001	<0.0001	0.1164
Phytase		<0.0001	<0.0001	0.0003
Ca:AvP × phytase		<0.0001	0.0176	0.0406
Ca:AvP (linear)[3]		<0.0001	<0.0001	<0.0001
Ca:AvP (quadratic)[3]		0.0077	0.0141	0.1088

[1]Each value represents the mean of 6 replicates (8 birds per replicate). FTU = phytase units.

[2]Pooled SEM.

[3]Orthogonal polynomial contrasts were used to assess the significance of linear or quadratic models to describe the response in the dependent variable to Ca:AvP level with or without added phytase.

Energy, Phytate, P, and Ca Digestibility

The effects of dietary Ca:AvP ratios and microbial phytase on bone ash, energy, phytate, P, and Ca digestibility are summarized in Table 3. Phytase supplementation increased ($P < 0.05$) phytate degradation, energy digestibility, and P digestibility. Dietary Ca:AvP ratio had no effect ($P > 0.05$) on energy digestibility but significantly influenced phytate degradation and P digestibility. Phytase supplementation and dietary Ca:AvP ratio had no effect ($P > 0.05$) on Ca digestibility. There was no significant interaction between dietary Ca:AvP ratio and phytase supplementation on phytate degradation, energy, P, and Ca digestibility. However, there was a significant interaction between dietary Ca:AvP ratio and phytase supplementation for bone ash. Increasing the dietary Ca:AvP ratio in the absence of phytase reduced bone ash; conversely, in the presence of phytase, bone ash was increased at higher Ca:AvP ratios. Increasing dietary Ca:AvP ratio led to a significant quadratic decrease in phytate degradation and significant linear decreases in P digestibility and bone ash.

AA Digestibility

The effects of dietary Ca:AvP ratio and phytase supplementation on individual AA are summarized in Table 4. Phytase supplementation improved ($P < 0.05$) digestibility of all AA. Dietary Ca:AvP ratio influenced ($P < 0.05$) digestibility of all AA apart from Arg, Gly, Tyr, Val, and Cys. Increasing dietary Ca:AvP ratio reduced ($P < 0.05$) AA digestibility in a linear manner. Mean AA digestibility was improved ($P < 0.05$) by phytase supplementation (Figure 2).

Regression for AA Digestibility

The AA digestibility significantly increased in a curvilinear manner with increasing phytate degradation ($y = -0.0031x^2 + 0.4942x + 64.667$, $R^2 = 0.74$, $P < 0.05$), Figure 3.

Gastrointestinal pH

The effect of dietary Ca:AvP ratio and microbial phytase supplementation on gastrointestinal pH is summarized in Table 5. Diets receiving phytase exhibited a significant increase in gizzard and ileum pH. The Ca:AvP ratio only influenced ($P < 0.05$) the pH of gizzard digesta. Increasing dietary Ca:AvP ratio significantly increased gizzard pH in a linear manner. There was no effect ($P > 0.05$) of dietary Ca:AvP ratio on the pH of the other regions of the gastrointestinal tract: crop, duodenum, and ileum. There was a significant interaction between dietary Ca:AvP ratio and phytase

Table 3. Effect of varying dietary Ca:available P (AvP) ratios and microbial phytase on energy digestibility (%), phytate degradation (%), Ca and P digestibility (%), and bone ash (%) of broilers fed a corn/soy-based diet[1]

Item	Phytase (FTU/kg)	Gross energy digestibility (%)	Phytate degradation (%)	Ca digestibility (%)	P digestibility (%)	Bone ash (%)
Ca:AvP ratio						
1.43	0	67.4	51.4	53.8	61.5	43.9
2.14	0	64.6	40.4	50.4	52.5	41.1
2.86	0	66.8	43.7	54.9	55.3	39.3
3.57	0	65.8	39.8	61.8	51.2	38.0
1.43	1,000	74.6	88.4	56.1	80.4	45.1
2.14	1,000	74.1	75.2	40.7	72.4	47.6
2.86	1,000	69.7	76.2	49.3	67.6	48.6
3.57	1,000	73.9	75.9	58.6	67.6	46.8
SEM[2]		1.5	2.6	3.7	2.2	0.7
Main effect						
Ca:AvP ratio						
1.43		71.0	69.9	55.0	71.0	44.5
2.14		69.4	57.8	45.6	62.5	44.4
2.86		68.3	59.9	52.1	61.5	43.9
3.57		69.9	57.9	60.2	59.2	42.4
Phytase (FTU/kg)						
0		66.2[b]	43.8[b]	55.2	55.1[b]	40.6
1,000		73.1[a]	78.9[a]	51.2	71.9[a]	47.0
P-value						
Ca:AvP		0.3795	0.0006	0.0932	<0.0001	0.005
Phytase		<0.0001	<0.0001	0.1676	<0.0001	<0.0001
Ca:AvP × phytase		0.6935	0.7821	0.6362	0.2759	<0.0001
Ca:AvP (linear)[3]		0.3691	0.0003	0.0613	<0.0001	0.0268
Ca:AvP (quadratic)[3]		0.1514	0.0059	0.0016	0.0568	0.2950

[a,b]Means in a column not sharing a common superscript are different ($P < 0.05$).

[1]Each value represents the mean of 6 replicates (8 birds per replicate). FTU = phytase units.

[2]Pooled SEM.

[3]Orthogonal polynomial contrasts were used to assess the significance of linear or quadratic models to describe the response in the dependent variable to Ca:AvP level with or without added phytase.

Table 4. Apparent ileal amino acid (AA) digestibility (%) of broilers fed a corn/soy-based diet with varying dietary Ca:available P (AvP) ratios supplemented with or without microbial phytase[1]

Item	Phytase (FTU/kg)	His	Ser	Arg	Gly	Asp	Glu	Thr	Ala	Pro	Lys	Tyr	Met	Val	Iso	Leu	Phe	Cys	Mean AA
Ca:AvP ratio																			
1.43	0	75.6	72.4	85.6	68.9	79.9	83.8	69.7	73.7	74.9	84.7	77.8	89.5	68.5	75.2	75.6	76.9	57.5	78.0
2.14	0	70.4	65.3	79.3	62.1	73.9	78.6	62.7	67.2	70.4	77.1	73.4	87.4	64.2	69.4	69.9	71.6	51.8	72.3
2.86	0	75.7	70.9	84.9	69.2	76.9	82.2	68.8	72.9	73.7	84.3	77.8	90.4	69.4	75.1	74.5	75.8	54.1	77.0
3.57	0	70.2	65.1	79.3	63.1	71.5	78.4	63.2	65.7	69.8	76.2	73.0	84.8	65.5	68.7	68.9	70.6	53.7	71.8
1.43	1,000	84.6	84.3	90.8	79.6	87.9	89.9	79.2	83.7	83.6	89.6	85.9	93.6	81.4	85.0	85.5	86.3	72.6	86.4
2.14	1,000	83.0	82.0	90.2	77.7	85.9	88.9	77.4	82.0	82.3	89.5	85.1	93.7	77.9	83.0	83.5	84.3	69.3	84.9
2.86	1,000	80.5	79.2	89.7	74.1	83.8	87.0	73.9	78.0	79.3	88.6	82.9	92.5	75.0	80.0	80.3	81.3	65.1	82.4
3.57	1,000	80.3	78.1	89.3	74.6	82.5	87.0	75.4	79.0	79.7	87.9	83.7	91.5	75.2	80.4	80.9	81.6	67.1	82.4
SEM[2]		1.70	2.08	1.37	2.16	1.52	1.23	2.04	1.94	1.50	1.58	1.55	1.26	2.13	1.86	1.78	1.66	2.33	1.55
Main effect																			
Ca:AvP ratio																			
1.43		80.1	78.4	88.2	74.3	83.9	86.8	74.4	78.7	79.2	87.2	81.9	91.6	75	80.1	80.6	81.6	65.1	82.2
2.14		76.7	73.7	84.8	69.9	79.9	83.8	70.0	74.6	76.4	83.3	79.2	90.6	71.0	76.2	76.7	78.0	60.6	78.6
2.86		78.1	75.0	87.3	71.6	80.4	84.6	71.4	75.4	76.5	86.4	80.3	91.5	72.2	77.5	77.4	78.5	59.6	79.7
3.57		75.2	71.6	84.3	68.9	77.0	82.7	69.3	72.4	74.7	82.1	78.4	88.1	70.4	74.5	74.9	76.1	60.4	77.1
Phytase (FTU/kg)																			
0		73.0[b]	68.4[b]	82.3[b]	65.8[b]	75.6[b]	80.7[b]	66.1[b]	69.9[b]	72.2[b]	80.6	75.5[b]	88.0[b]	66.9[b]	72.1[b]	72.2[b]	73.7[b]	54.3[b]	74.8[b]
1,000		82.1[a]	80.9[a]	90.0[a]	76.5[a]	85.0[a]	88.2[a]	76.5[a]	80.7[a]	81.2[a]	88.9	84.4[a]	92.8[a]	77.4[a]	82.1[a]	82.6[a]	83.4[a]	68.5[a]	84.0[a]
P-value																			
Ca:AvP		0.0238	0.0078	0.0656	0.0505	0.0002	0.0072	0.0436	0.0082	0.0083	0.0402	0.0709	0.0291	0.0688	0.0173	0.0079	0.0051	0.0501	0.0085
Phytase		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Ca:AvP × phytase		0.6753	0.7033	0.4103	0.5587	0.6807	0.8126	0.9077	0.9827	0.7708	0.3059	0.8978	0.6813	0.1946	0.8134	0.8808	0.8292	0.4333	0.9883
Ca:AvP (linear)[3]		0.0240	0.0077	0.0676	0.0508	0.0002	0.0070	0.0423	0.0082	0.0082	0.0424	0.0707	0.0268	0.0725	0.0172	0.0077	0.0050	0.0454	0.0090
Ca:AvP (quadratic)[3]		0.8249	0.6873	0.8409	0.6345	0.7724	0.5401	0.4516	0.7228	0.5925	0.8421	0.7810	0.2176	0.4887	0.7539	0.5942	0.6183	0.1093	0.6640

[a,b]Means in a column not sharing a common superscript are different ($P < 0.05$).

[1]Each value represents the mean of 6 replicates (8 birds per replicate). FTU = phytase units.

[2]Pooled SEM.

[3]Orthogonal polynomial contrasts were used to assess the significance of linear or quadratic models to describe the response in the dependent variable to Ca:AvP level with or without added phytase.

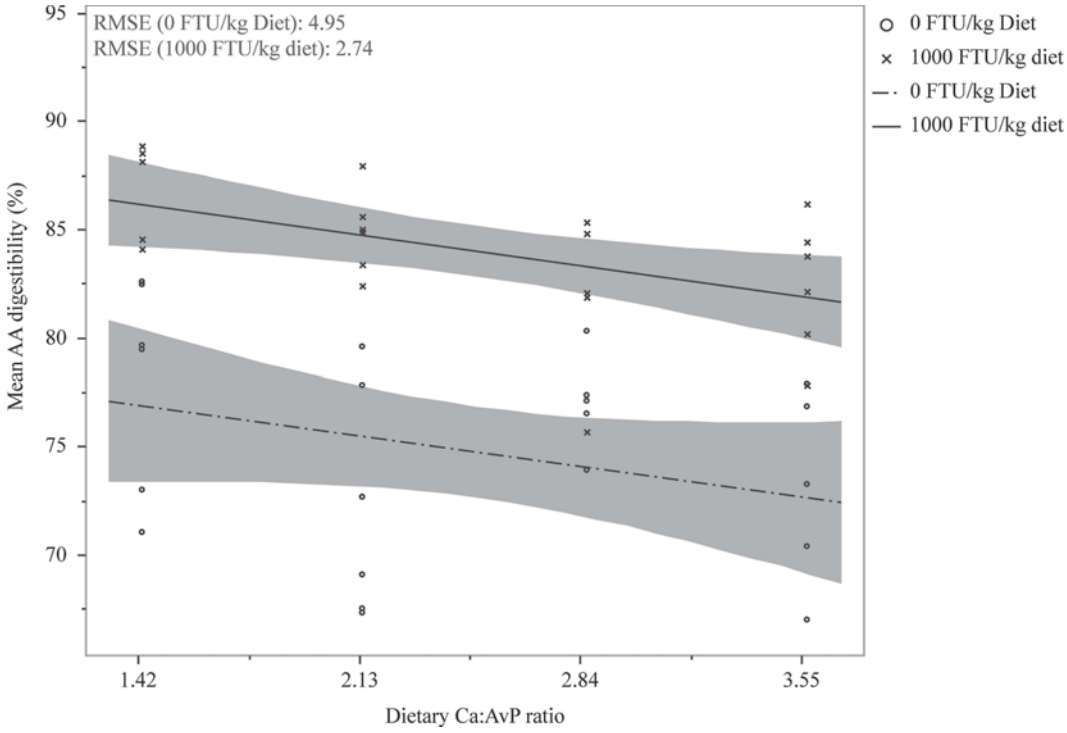

Figure 2. Regression of mean amino acid (AA) digestibility in broilers fed diets containing different Ca:available P (AvP) ratios, in the presence and absence of a supplementary microbial phytase from *Buttiauxella*. FTU = phytase units, RMSE = root mean square error.

supplementation on duodenal pH whereby in the absence of phytase an increase in dietary Ca:AvP ratio did not have an effect on duodenal pH; conversely, in the presence of phytase an increase in dietary Ca:AvP resulted in an increase in duodenal pH.

DISCUSSION

In the current study phytase supplementation improved WG at all Ca:AvP ratios. The detrimental effect on WG from increasing Ca:AvP ratios was greater in the unsupplemented diets, compared with the phytase-supplemented diets, as noted by the significant interaction between dietary Ca:AvP ratio and phytase supplementation. This can likely be attributed to diets with no phytase being more P deficient, and a negative effect of Ca on Phytate P utilization. Tamim et al. (2004) showed that in the absence of dietary Ca, broilers were able to use 69.2% of phytate P by the terminal ileum. However, this was reduced to 25.4% when Ca levels were increased to 0.5%. Phytase supplementation mitigated the negative effect of increasing Ca:AvP ratio on

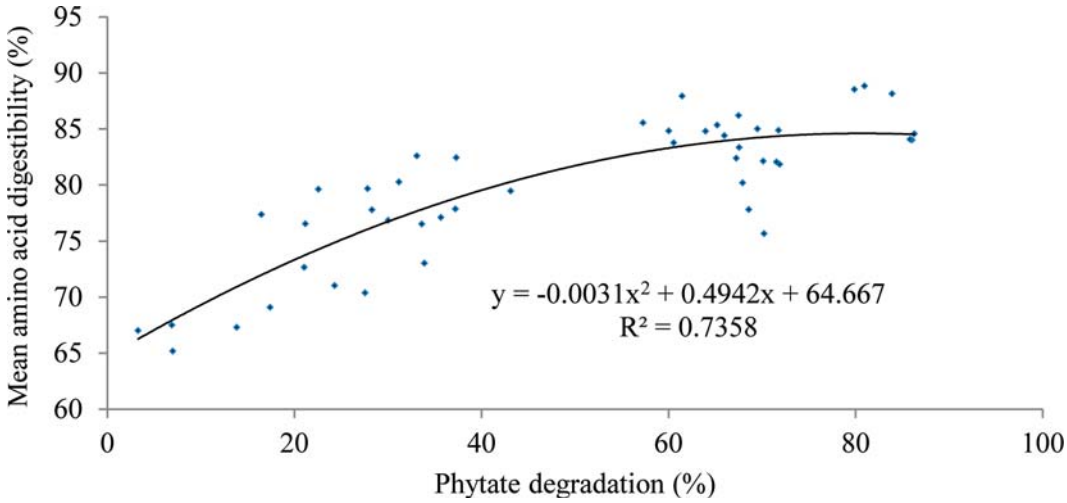

Figure 3. Regression of phytate degradation and mean amino acid digestibility in broilers fed diets containing different Ca:available P (AvP) ratios, in the presence and absence of a supplementary microbial phytase from *Buttiauxella*.

Table 5. Effect of varying dietary Ca:available P (AvP) ratios and microbial phytase on pH of the gastrointestinal tract in broilers fed a corn/soy-based diet[1]

Item	Phytase (FTU/kg)	Crop pH	Gizzard pH	Duodenum pH	Ileum pH
Ca:AvP ratio					
1.43	0	4.5	2.8	5.9	5.5
2.14	0	4.3	3.2	6.0	5.2
2.86	0	4.8	3.2	5.9	5.3
3.57	0	4.5	3.3	5.7	5.4
1.43	1,000	4.7	2.5	5.7	6.4
2.14	1,000	4.7	2.4	5.7	6.4
2.86	1,000	4.8	2.4	5.6	6.7
3.57	1,000	4.9	3.2	5.9	6.2
SEM[2]		0.2	0.2	0.1	0.2
Main effect					
Ca:AvP ratio					
1.43		4.6	2.6	5.8	5.9
2.14		4.5	2.8	5.8	5.8
2.86		4.8	2.8	5.8	6.0
3.57		4.7	3.2	5.8	5.8
Phytase (FTU/kg)					
0		4.5	2.6[b]	5.7	5.4[b]
1,000		4.8	3.2[a]	5.9	6.4[a]
P-value					
Ca:AvP		0.0975	0.0003	0.5999	0.4098
Phytase		0.0607	<0.0001	0.0552	<0.0001
Ca:AvP × phytase		0.2203	0.7385	0.0384	0.3655
Ca:AvP (linear)[3]		0.3364	0.0006	0.8111	0.7955
Ca:AvP (quadratic)[3]		0.9044	0.3508	0.9723	0.9389

[a,b]Means in a column not sharing a common superscript are different ($P < 0.05$).

[1]Each value represents the mean of 6 replicates (8 birds per replicate). FTU = phytase units.

[2]Pooled SEM.

[3]Orthogonal polynomial contrasts were used to assess the significance of linear or quadratic models to describe the response in the dependent variable to Ca:AvP level with or without added phytase.

FI and FCR at all dietary Ca:AvP ratios. These data may be explained by the higher nutrient digestibility observed in this study when the diets were supplemented with phytase. Improved broiler performance with the addition of phytase to low P diets is to be expected (Selle and Ravindran, 2007). In contrast, Powell et al. (2011) reported improved WG and FI with no effect on FCR when the diet was supplemented phytase. Increasing Ca:AvP ratio reduced FI in the current study, which is in agreement with previous reports (Powell et al., 2011; Delezie et al., 2012).

In the current study, increasing the Ca:AvP ratio reduced phytate degradation quadratically and P digestibility linearly. One phytate molecule can bind up to 5 Ca atoms and the extent of this complex formation has been reported to be driven by gut pH and molar ratios of the 2 components (Selle et al., 2009). Previous reports have shown that the degree of phytate degradation was highly dependent on the dietary Ca level (Tamim et al., 2004; Plumstead et al., 2008; Selle et al., 2009). Plumstead et al. (2008) reported a linear reduction in ileal phytate P degradation by 71%, when increasing dietary Ca level from 4.7 to 11.6 g/kg in broiler diets. Similar results were reported by Tamim et al. (2004) in both in vitro and in vivo studies. These researchers found that dietary Ca at a level as low as 0.1% reduced phytate-P hydrolysis at pH 6.5 in vitro. This effect has been proven in vivo by adding Ca at a level of 0.5% to the diet, which resulted in a reduction of phytate-P disappearance from 69.2 to 25.4%. The negative effects of high levels of Ca on phytate degradation and P digestibility may be explained by the formation of Ca-Phytate complexes and the increase in the pH of the proximal digestive tract (Selle et al., 2009). The reason for the quadratic response to phytate degradation in this study is not clear, but may be due to saturation of the phytate binding sites with Ca ions, in which case increasing Ca concentration further would have no additional effect.

Phytase supplementation increased phytate degradation and improved P digestibility at all Ca:AvP ratios. In contrast to the findings of the current study, other studies have shown that phytase effects on P retention were reduced at higher than optimal Ca:P ratios (Qian et al. 1997). These researchers suggested that the extra Ca may directly suppress phytase activity by competing for the active sites of the enzymes. The inconsistent results of these data compared with that reported previously in the literature may be explained, partly, by the differences in Ca:AvP ratios tested, phytase level used, and differences in the characteristics of the phytase enzymes used. The source of phytase used in these earlier studies was of fungal origin with a higher pH optimum, which may be more prone to Ca inhibition. In their review, Selle et al. (2009) hypothesized that as Ca-phytate complexes were mainly formed in the

small intestine and exogenous phytases of bacterial origin would be more active in more proximal segments of the gut where the pH was closer to the optimum pH of the phytase. Further, phytate would be less likely to bind Ca and the efficacy of these bacterial phytases would be influenced to a lesser extent by Ca–phytate complexes.

At low Ca:AvP ratios the response to phytase was lower compared with higher Ca:AvP ratios. These data suggest that at a low Ca:AvP ratio, broilers were able to use phytate P better and therefore maintain bone ash levels similar to those fed the phytase supplemented diet. Similar results were observed by Rousseau et al. (2012) who showed a tendency for interaction between Ca, nonphytate P (**nPP**), and phytase supplementation, such that a lower response to phytase at lower Ca:nPP levels were observed. In contrast, Powell et al. (2011) reported that phytase supplementation of a high Ca diet increased bone breaking strength, bone weight, ash weight, and percentage of tibia ash. Interestingly, in the present study, there was significantly higher Ca digestibility observed at the low Ca:AvP (1.43) compared with the Ca:AvP (2.14). This suggested a higher efficiency of Ca utilization at lower Ca levels, which may be caused by upregulation of Ca transporters at Ca levels below the bird's requirement (Li et al., 2012).

Phytase addition improved the digestibility of all measured AA. There was a strong correlation between AA digestibility and degree of phytate degradation. These positive effects of phytase supplementation on AA digestibility are supported by previous studies (Rutherfurd et al., 2002; Ravindran et al., 2006). Increasing dietary Ca:AvP ratio significantly decreased the digestibility of most AA. The exact mechanism of the effects of varying Ca:AvP ratio on AA acid digestibility remains to be determined, but may be related to a reduction in phytate hydrolysis and increased formation of Ca-phytate or Ca-protein binary complexes, increased endogenous losses, an increase in the pH of the proximal digestive tract, or a combination of these. Calcium may also interact with protein (Selle et al., 2012), which may also explain the lower AA digestibility at higher Ca levels. It should be noted that in the current study, birds were killed by cervical dislocation, which has been suggested to cause mixing of the digesta between intestinal segments and thus influence ileal digestibility. However, to the authors' knowledge, this is, so far, unsupported in the scientific literature.

Digesta pH was higher in all segments of the digestive tract when diets contained phytase. Increased pH in the gizzard, duodenum, jejunum, and ileum was also reported by Walk et al. (2012) when diets were supplemented to provide 5,000 FTU/kg of feed. Woyengo et al. (2010) reported that phytate decreased jejunal digesta pH in pigs. These authors suggested that phytate reduced pepsin activity in the stomach and resulted in higher secretion of HCl and reduced pH of the digesta in the stomach and the upper part of the small intestine. Therefore, it is plausible to suggest that in the current study, the addition of a bacterial phytase with a low pH optimum (Shukun Yu, DuPont Industrial Biosciences, Aarhus, Denmark, personal communication) would have degraded the phytate in the area of the proventriculous, reducing the production of the HCl from the proventriculus, which may have caused the increase in the digesta pH. Increasing dietary Ca:AvP ratio increased digesta pH in the gizzard. These results were in agreement with previous reports showing increased digesta pH due to adding Ca as limestone due to limestone's high acid binding capacity (Selle et al., 2009).

Our results supported previous observations that lowering dietary Ca:AvP positively affected phytate and P digestibility. However, the comparatively small negative effects on the efficacy of phytase when the Ca:AvP increased above 2.14 suggest that bacterial phytases that hydrolyze phytate at low pH in the proximal intestinal tract may be less prone to inhibition by higher dietary Ca levels. The positive effects of phytate hydrolysis on AA digestibility support the negative effect that dietary phytate can have on the digestibility of nutrients other than P.

REFERENCES

Amerah, A. M., and V. Ravindran. 2009. Influence of maize particle size and microbial phytase supplementation on the performance, nutrient utilisation and digestive tract parameters of broiler starters. Anim. Prod. Sci. 49:704–710.

Angel, R., N. M. Tamim, T. J. Applegate, A. S. Dhandu, and L. E. Ellestad. 2002. Phytic acid chemistry: influence on phytin –phosphorus availability and phytase efficacy. J. Appl. Poult. Res. 11:471–480.

AOAC (Association of Official Analytical Chemists). 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Washington, DC.

Cohen, S. A., and K. M. DeAntonis. 1994. Applications of amino acid analysis derivatisation with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate: Analysis of feed grains, intravenous solutions and glycoproteins. J. Chromatog. 661:25–34.

Delezie, E., L. Maertens, and G. Huyghebaert. 2012. Consequences of phosphorus interactions with calcium, phytase, and cholecalciferol on zootechnical performance and mineral retention in broiler chickens. Poult. Sci. 91:2523–2531.

Latta, M., and M. Eskin. 1980. A simple and rapid colorimetric method for phytate determination. J. Agric. Food Chem. 28:1313–1315.

Li, J., J. Yuan, Y. Guo, Q. Sun, and X. Hu. 2012. The influence of dietary calcium and phosphorus imbalance on intestinal NaPi-IIb and calbindin mRNA expression and tibia parameters of broilers. Asian-australas. J. Anim. Sci. 25:552–558.

Plumstead, P. W., A. B. Leytem, R. O. Maguire, J. W. Spears, P. Kwanyuen, and J. Brake. 2008. Interaction of calcium and phytate in broiler diets. 1. Effects on apparent prececal digestibility and retention of phosphorus. Poult. Sci. 87:449–458.

Powell, S., T. D. Bidner, and L. L. Southern. 2011. Phytase supplementation improved growth performance and bone characteristics in broilers fed varying levels of dietary calcium. Poult. Sci. 90:604–608.

Qian, H., E. T. Kornegay, and D. M. Denbow. 1997. Utilization of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol, and the calcium:total phosphorus ratio in broiler diets. Poult. Sci. 76:37–46.

Ravindran, V., S. Cabahug, G. Ravindran, and W. L. Bryden. 1999. Influence of microbial phytase on apparent ileal amino acid digestibility in feedstuffs for broilers. Poult. Sci. 78:699–706.

Ravindran, V., P. C. H. Morel, G. G. Partridge, M. Hruby, and J. S. Sands. 2006. Influence of an *E. coli*-derived phytase on nutrient

utilization in broilers fed starter diets containing varying concentrations of phytic acid. Poult. Sci. 85:82–89.

Rousseau, X., M. P. Létourneau-Montminy, N. Même, M. Magnin, Y. Nys, and A. Narcy. 2012. Phosphorus utilization in finishing broiler chickens: effects of dietary calcium and microbial phytase. Poult. Sci. 91:2829–2837.

Rutherfurd, S. M., T. K. Chung, and P. J. Moughan. 2002. The effect of microbial phytase on ileal phosphorus and amino acid digestibility in broiler chickens. Br. Poult. Sci. 44:598–606.

Selle, P. H., A. J. Cowieson, N. P. Cowieson, and V. Ravindran. 2012. Protein-phytate interactions in pig and poultry nutrition: A reappraisal. Nutr. Res. Rev. 25:1–17.

Selle, P. H., A. J. Cowieson, and V. Ravindran. 2009. Consequences of calcium interactions with phytate and phytase for poultry and pigs. Livest. Sci. 124:126–141.

Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol. 135:1–41.

Selle, P. H., V. Ravindran, R. A. Caldwell, and W. L. Bryden. 2000. Phytate and phytase: Consequences for protein utilisation. Nutr. Res. Rev. 13:255–278.

Tamim, N. M., R. Angel, and M. Christman. 2004. Influence of dietary calcium and phytase on phytate phosphorus hydrolysis in broiler chickens. Poult. Sci. 83:1358–1367.

Tran, T. T., R. Hatti-Kaul, S. Dalsgaard, and S. Yu. 2011. A simple and fast kinetic assay for phytases using phytic acid-protein complex as substrate. Anal. Biochem. 410:177–184.

Walk, C. L., M. R. Bedford, and A. P. McElroy. 2012. Influence of limestone and phytase on broiler performance, gastrointestinal pH, and apparent ileal nutrient digestibility. Poult. Sci. 91:1371–1378.

Woyengo, T. A., O. Adeola, and C. M. Nyachoti. 2010. Gastrointestinal digesta pH, pepsin activity and soluble mineral concentration responses to dietary phytic acid and phytase in piglets. Livest. Sci. 134:91–93.