Interaction of Calcium and Phytate in Broiler Diets. 1. Effects on Apparent Prececal Digestibility and Retention of Phosphorus[1]

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ABSTRACT Phytate P utilization from soybean meal (SBM) included in broiler diets has been shown to be poor and highly dependent on dietary Ca intake. However, the effect of Ca on P utilization and on the optimal ratio of Ca to nonphytate P (Ca:NPP) when diets contained varying levels of phytate has not been clearly shown and was the objective of this research. A factorial treatment structure was used with 4 dietary Ca levels from 0.47 to 1.16% and 3 levels of phytate P (0.28, 0.24, and 0.10%). Varying dietary phytate P levels were obtained by utilizing SBM produced from 3 varieties of soybeans with different phytate P concentrations. Ross 508 broiler chicks were fed 1 of 12 diets from 16 to 21 d of age. Excreta were collected from 16 to 17 d and from 19 to 20 d of age and ileal digesta was collected at 21 d of age. Apparent prececal P digestibility decreased when dietary Ca concentration increased and was higher when diets contained low-phytate SBM. The apparent digestibility of Ca and percentage of phytate P hydrolysis at the distal ileum were not reduced when dietary phytate P concentration increased. Including low-phytate SBM in diets reduced total P output in the excreta by 49% compared with conventional SBM. The optimum ratio of Ca:NPP that resulted in the highest P retention and lowest P excretion was 2.53:1, 2.40:1, and 2.34:1 for diets with 0.28, 0.24, and 0.10% phytate P. These data suggested that increased dietary Ca reduced the extent of phytate P hydrolysis and P digestibility and that the optimum Ca:NPP ratio at which P retention was maximized was reduced when diets contained less phytate P.

Key words: broiler, phosphorus, soybean meal, phytate, environment

2008 Poultry Science 87:449–458
doi:10.3382/ps.2007-00231

INTRODUCTION

Broiler diets have been mainly made from plant-based feed ingredients that, in addition to serving as dietary sources of starch (energy), protein, and fat, also contribute substantially to the total dietary P content. However, over 60% of the total P from conventional ingredients such as corn, wheat, and soybean meal (**SBM**) has been reported to be present as salts of phytic acid (*myo*-inositol 1,2,3,4,5,6 hexakis dihydrogen phosphate), also known as phytate, which has been reported to be poorly digested by monogastric animals (Nelson et al., 1968a; Raboy et al., 1984). The utilization of phytate P in broiler diets, measured as disappearance of phytate P from the distal ileum, was also highly variable, ranging from 25.4 to 69.2% (Rutherford et al., 2002; Tamin et al., 2004). A considerable part of the large variation in the extent of phytate P hydrolysis in the small intestine was shown to be caused by differences in the dietary Ca concentration (Tamin et al., 2004). A high dietary Ca concentration has been thought to reduce hydrolysis of phytate P by endogenous and exogenous phytase enzymes as a result of the formation of insoluble Ca-phytate complexes (Nelson, 1980; Sands et al., 2003; Tamin and Angel, 2003; Tamin et al., 2004). Furthermore, a high ratio of dietary Ca to nonphytate P (**NPP**) in the diet was also shown to antagonize the digestibility and absorption of inorganic soluble forms of P due to increased precipitation of insoluble Ca:P complexes (Hurwitz and Bar, 1971).

To improve the utilization of P from SBM, low-phytate (**LP**) varieties of soybeans have been selectively bred that have similar nutritive value and total P concentrations but contained considerably reduced levels of phytate P (Wilcox et al., 2000; Raboy, 2002). However, reducing the dietary phytate P concentration by including LP grains

Received June 5, 2007.
Accepted November 23, 2007.
[1]The use of trade names in this publication does not imply endorsement of the products mentioned nor criticism of similar products not mentioned.
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Table 1. Analyzed composition of 3 soybean meals (SBM) with different phytate concentrations[1,2]

SBM source	CP	Lys	Total P	IP3[3]	IP4[3]	IP5[3]	IP6[3]	Total IP[3]	Phytate P[4]	Phytate P[4] (% of total P)
	mg/g									
HP Prolina	489.6	3.09	7.80	0.99	0.73	4.71	18.17	24.60	5.12	66
Commercial	503.3	3.09	6.10	1.12	0.21	2.24	16.09	19.66	4.53	74
Low phytate	502.5	3.10	6.10	1.52	0.07	0.85	5.35	7.78	1.51	25

[1]HP = high phytate; IP = inositol phosphate esters.

[2]Nutrient composition based on analyzed nutrient values and standardized to 88% DM.

[3]IP esters analyzed by HPLC (Kwanyuen and Burton, 2005).

[4]Phytate P represented the P content as IP6, calculated as 0.2818 × IP6 (Angel et al., 2002).

would also presumably increase the *M*-ratio of Ca:phytate P in the intestine, which would influence the fate of phytate P (Wise, 1983). Early findings by Nelson et al. (1968b) demonstrated a 50% reduction in the Ca requirement for maximum bone ash when the dietary phytate P content was reduced in broiler diets. This was hypothesized to be caused by the ability of 1% phytate in a diet to chelate 0.36% Ca, thus decreasing the amount of Ca absorbed and increasing the dietary Ca concentration required for maximum bone ash, which would also alter the optimum dietary ratio of Ca:NPP. The potential effect of dietary phytate on the optimum ratio of Ca:P was further supported by Van der Klis and Versteegh (1996), who suggested that the optimum ratio of Ca:available P in broilers was reduced when diets contained less phytate. However, these authors did not quantify the effects of dietary Ca on the intestinal digestibility and overall retention of Ca and P from phytate when diets contained varying levels of both Ca and phytate.

Therefore, the objective of this study was to quantify the effects of dietary Ca on Ca and P absorption from the intestine, overall P retention, and the optimum ratio of Ca:P when diets contained different phytate P concentrations from inclusion of either high-phytate (**HP**), conventional, or LP varieties of SBM.

MATERIALS AND METHODS

Birds and Management

All animal work was approved by the Institutional Animal Care and Use Committee of North Carolina State University. Broiler chicks were hatched from eggs obtained from the resident flock of Ross 344 × 508 broiler breeders. Chicks were feather-sexed at hatching, and 816 male chicks were permanently identified with neck tags and 17 chicks randomly allocated to each of 48 electrically heated battery brooders located within 2 environmentally controlled brooding rooms. To reduce vertical temperature and lighting differences among the cages in the battery brooders, only the middle 4 tiers of cages in each 6-tier battery brooder were utilized. Brooding temperatures within cages were initially set at 33°C and reduced gradually to 25°C by 21 d of age. From 1 to 15 d, all birds had ad libitum access to feed and water and received a standard corn-soy broiler starter diet containing 3,150 kcal of ME/kg, 23.0% CP, 0.90% Ca, and 0.45% NPP.

Dietary Treatments

To obtain practical broiler diets with different phytate P concentrations, SBM made from 3 different cultivars of soybeans that differed in their natural phytate content was utilized (Table 1). The HP Prolina SBM (Burton et al., 1999) and the LP SBM (Wilcox et al., 2000) were processed from soybeans selected for either improved protein or LP P content. A commercial SBM with similar protein and amino acid concentration and intermediate in its phytate concentration was selected as a control. Further, to increase the range of phytate P in the final diets, degermed dehulled (**DGDH**) corn with a low level of phytate P was used in the formulations.

Three basal diets that differed in their source of SBM were formulated to contain similar nutrient profiles with the exception of dietary Ca (Table 2). Finely ground limestone was added to each of the basal diets at the expense of washed sand to obtain graded levels of dietary Ca (0.47, 0.70, 0.93, and 1.16%). Titanium dioxide and celite were included in diets as indigestible markers to allow the calculation of apparent nutrient digestibility and overall retention. To further reduce differences between diets, the soy oil added to diets was held constant at 6.2%, and the formulated moisture content was fixed at 11.5% by varying the inclusion of water. The analyzed phytate P levels were 0.28, 0.24, and 0.10% for the HP Prolina, commercial, and LP SBM diets, respectively (Table 2). All diets were mixed in two 25-kg batches in a bakery-style vertical mixer, and each batch was sampled and analyzed individually.

To reduce variation in chick BW between pens, all chicks were individually weighed at 12 d, and chicks with extreme BW were eliminated so that 13 chicks per pen with a mean chick BW of 342 ± 12 g remained. At 15 d of age, birds were fasted for 16 h after which the 12 experimental diets were each assigned to 4 pens of 13 birds and fed from 16 to 21 d of age.

Sample Collection and Analyses

Excreta were collected from metal pans lined with clean plastic beneath each cage during each of two 24-h periods. Excreta collection I (16 to 17 d) commenced after birds had been fasted for 16 h and placed on experimental treatments. Excreta collection II (19 to 20 d) commenced at 19 d after an adaptation period of 72 h. After each

Table 2. Formulation and calculated analyses of the basal diets

Ingredients	Soybean meal source[1]		
	HP Prolina	Commercial	Low phytate
	%		
DGDH corn[2]	45.80	46.74	46.65
Soybean meal	40.06	39.32	36.44
Monobasic calcium phosphate	1.21	1.09	0.59
Limestone[3]	0.34 to 2.15	0.44 to 2.25	0.64 to 2.45
Lys HCl	0.00	0.04	0.04
DL-Met	0.27	0.28	0.25
L-Thr	0.01	0.05	0.09
Premixes[4]	0.55	0.55	0.55
Sodium chloride	0.50	0.50	0.50
TiO_2 marker	0.40	0.40	0.40
Celite marker	2.00	2.00	2.00
Soybean oil	6.20	6.20	6.20
Filler (washed sand)[3]	0.10 to 1.91	0.43 to 2.24	0.76 to 2.56
Water[5]	0.74	0.16	3.09
Calculated nutrients[6]			
Moisture,[5] %	11.50	11.50	11.50
ME, kcal/g	3.06	3.06	3.06
CP, %	230.0	230.0	230.0
Lys, %	1.33	1.33	1.33
Met + Cys, %	0.97	0.97	0.97
Thr, %	0.88	0.88	0.88
Arg, %	1.63	1.55	1.50
Ca,[3] %	0.47 to 1.16	0.47 to 1.16	0.47 to 1.16
Total P, %	0.65	0.54	0.42
Nonphytate P,[7] %	0.35	0.35	0.35
Ca: nonphytate P ratio	1.4 to 3.3	1.4 to 3.3	1.4 to 3.3
Phytate P, %	0.28	0.24	0.10
Sodium, g/kg	0.20	0.20	0.20
DEB,[7] mEq/kg	27.47	26.84	24.98

[1]Three different sources of soybean meal that contained similar CP and amino acids but varied in phytate P were used in formulation. HP = high phytate.

[2]Degermed dehulled (DGDH) corn (Moeser et al., 2002).

[3]The inclusion rate of limestone added to the basal diets was varied to provide 4 different levels of dietary Ca of 0.47, 0.70, 0.93, and 1.16%. Washed sand was used as an inert filler to adjust the volume of each diet formulation to 100%.

[4]Premixes provided the following per kilogram of diet: vitamin A, 13,200 IU; vitamin D_3, 4,000 IU; vitamin E, 66 IU; vitamin B_{12}, 39.6 μg; riboflavin, 13.2 mg; niacin, 110 mg; D-pantothenate, 22 mg; menadione (K_3), 4 mg; folic acid, 2.2 mg; thiamine, 4 mg; pyridoxine, 8 mg; D-biotin, 252 μg; selenium (as Na_2SeO_3), 0.30 mg; manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; cobalt, 1.0 mg; and choline chloride 1,200 mg.

[5]The moisture percentage of diets was adjusted to 11.5% with water.

[6]Nutrient compositions calculated from proximate analyses of all ingredients. Final diet composition was confirmed by proximate analyses of all diets.

[7]Dietary electrolyte balance (DEB) calculated as mEq (Na + K − Cl).

collection, the excreta from each pan were homogenized, subsampled, and immediately frozen. At 21 d of age, 10 chicks from each pen were weighed, killed by cervical dislocation, and the terminal 13 cm of ileum was removed 3 cm anterior to the ileo-cecal junction. The ileal digesta were gently expressed, pooled per cage, and frozen. Feeders were weighed at the start and end of excreta collection I and again at the end of excreta collection II to calculate the mean feed intake per bird.

Frozen samples of feed, ileal digesta, and excreta were lyophilized and finely ground before analyzing all samples in duplicate for total elements and phytate as follows: (i) total elements (Ca and P) were determined by microwave-assisted digestion of a 0.5-g dried sample with 8 mL of concentrated HNO_3 and 2 mL of 30% H_2O_2 (vol/vol) with all elements quantified using inductively coupled plasma optical-emission spectrometry detection (4300DV, Perkin-Elmer, Wellesley, MA), and (ii) phytate [inositol phosphate (**IP**) 6] was determined by acid extraction followed by HPLC analysis (Agilent HPLC 1100 series, Agilent Technologies, Wilmington, DE; Kwanyuen and Burton, 2005). The phytate-bound P was subsequently calculated by multiplying the analyzed phytate content by a factor of 0.2818, which represented the *M*-proportion of P in phytate (Angel et al., 2002). The TiO_2 of diets, ileal, and excreta samples was determined using the method of Short et al. (1996).

Calculations and Statistical Analyses

The apparent percentage of prececal nutrient digestibility ($PcND_{\%}$) and overall percentage of nutrient retention

Table 3. Calculated and determined nutrient analyses[1] of dietary treatments

Item		Soybean meal source											
		HP Prolina					Commercial				Low phytate		
Diet		1	2	3	4	5	6	7	8	9	10	11	12
		%											
Nutrient													
CP	Calculated	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00
	Determined	23.52	23.86	22.77	23.27	23.55	23.47	23.32	22.78	24.82	23.56	22.11	23.14
Lys	Calculated	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33
	Determined	1.33	1.33	1.28	1.33	1.35	1.37	1.41	1.33	1.37	1.30	1.25	1.30
Met + Cys	Calculated	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97
	Determined	0.99	0.97	0.97	0.98	0.99	0.99	1.00	1.00	0.99	0.92	0.92	0.91
Ca	Calculated	0.47	0.70	0.93	1.16	0.47	0.70	0.93	1.16	0.47	0.70	0.93	1.16
	Determined	0.51	0.79	0.92	1.13	0.51	0.65	0.75	1.05	0.55	0.74	1.30	1.40
Total P	Calculated	0.65	0.65	0.65	0.65	0.54	0.54	0.54	0.54	0.42	0.42	0.42	0.42
	Determined	0.66	0.66	0.67	0.69	0.57	0.56	0.55	0.51	0.44	0.42	0.41	0.40
Total IP esters	Determined	1.17	1.22	1.10	1.12	0.90	0.92	0.92	0.90	0.42	0.41	0.40	0.44
Phytate P[2]	Determined	0.28	0.29	0.26	0.27	0.24	0.24	0.24	0.23	0.10	0.10	0.10	0.11
NPP[3]	Calculated	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
	Determined	0.38	0.37	0.41	0.42	0.33	0.32	0.31	0.28	0.34	0.32	0.31	0.29
Ca:NPP ratio	Calculated	1.34	2.00	2.66	3.31	1.34	2.00	2.66	3.31	1.34	2.00	2.66	3.31
	Determined	1.34	2.14	2.24	2.69	1.55	2.03	2.42	3.75	1.62	2.31	4.19	4.83

[1]HP = high phytate; IP = inositol phosphate; NPP = nonphytate P.

[2]Calculated from the analyzed concentration of phytic acid (IP6) × 0.2818 (Angel et al., 2002).

[3]Defined as the difference between total P and phytate P.

($TNR_{\%}$), expressed as a percentage of DM nutrient concentration, were calculated using the index method based on the following equation of Dilger and Adeola (2006):

$$PcND_{\%} \text{ or } TNR_{\%} = 100 - [(TiO_{diet}/TiO_{out}) \times (Nut_{out}/Nut_{diet}) \times 100] \quad [1]$$

where TiO_{diet} = the initial TiO_2 concentration in the diet; Nut_{diet} = the initial dietary concentration of the nutrient being assessed; and TiO_{out} and Nut_{out} = the respective concentrations of either TiO_2 or nutrient in the ileal digesta or excreta, respectively.

To account for differences in dietary nutrient concentrations, the apparent amount (g) of nutrients absorbed per kilogram of DM intake (**DMI**) at the terminal ileum ($PcNA_g$), as well as the total retention of dietary nutrients per kilogram of DMI, (TNR_g), was calculated using the equation:

$$PcNA_g \text{ or } TNR_g \text{ (g/kg of DMI)} = PcNA_{\%} \text{ or } TNR_{\%} \times \text{nutrient intake/kg of DMI} \quad [2]$$

where $PcNA_{\%}$ or $TNR_{\%}$ = the percentage of nutrient digestibility or retention calculated in [1]. Further, the nutrient output per kilogram of DMI at the terminal ileum ($PcNE_g$,) or total nutrients excreted (TNE_g) per kilogram of DMI were calculated using the ratio of TiO_2 intake to TiO_2 output (Dilger and Adeola, 2006):

$$PcNE_g \text{ or } TNE_g \text{ (g/kg of DMI)} = NcE \times (TiO_{diet}/TiO_{out}) \quad [3]$$

where NcE = the concentration of the respective nutrient in the ileal digesta or excreta; TiO_{diet} = the initial TiO_2 concentration in the diet; and TiO_{out} = the concentration of TiO_2 in the ileal digesta or excreta. Finally, the sum of

$$PcNA_g + PcNE_g \text{ or } TNR_g + TNE_g = \text{dietary nutrient intake/kg of DMI.} \quad [4]$$

All data were analyzed using the mixed-models procedure (SAS Institute, 2004). There were 4 replicate cages per treatment arranged in a randomized complete block design. There were 4 blocks with 2 blocks of 4 cages located in each of 2 rooms. A cage of birds served as the experimental unit. Data were analyzed using a factorial effects model that included SBM source (3 levels), dietary Ca (4 levels), and all 2-way interactions as fixed effects with block as a random effect. Orthogonal polynomial contrasts were used to assess the significance of linear or quadratic models to describe the response in the dependent variable to increasing Ca level. Where appropriate, means separation was carried out using Tukey's honestly significant difference with an α level of 0.05. Further, the breakpoint in the dietary Ca dose at which a plateau in total P retention and output of P in the excreta was reached was determined using segmented regression models in PROC NLIN (SAS Institute, 2004). Correlation coefficients between specific independent and dependent variables were determined using the PROC CORR function of SAS (SAS Institute, 2004). Statements of statistical significance were based upon $P < 0.05$ unless otherwise stated.

RESULTS

Analysis of the SBM for total IP esters and phytate P is shown in Table 1. The majority of IP found was in the form of IP6, with decreasing concentrations of the lower

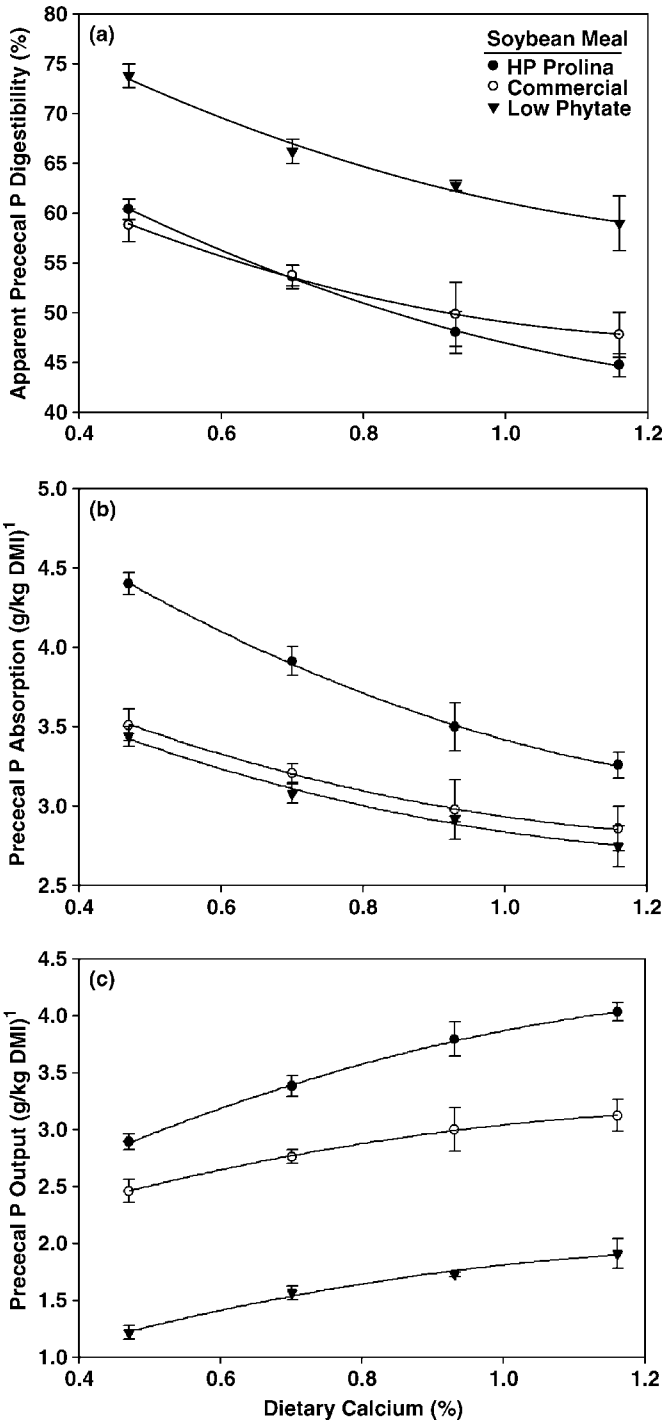

Figure 1. Effects of source of soybean meal with different phytate concentrations and dietary Ca level on apparent prececal P digestibility (a) and absorption (b) and output of phytate P at the distal ileum (c) of broilers at 21 d of age. HP = high phytate. [1]Prececal P absorption and prececal P output expressed in grams per kilogram of DM intake (DMI). Symbols represent the average response obtained from 4 replicate pens of 10 broilers each.

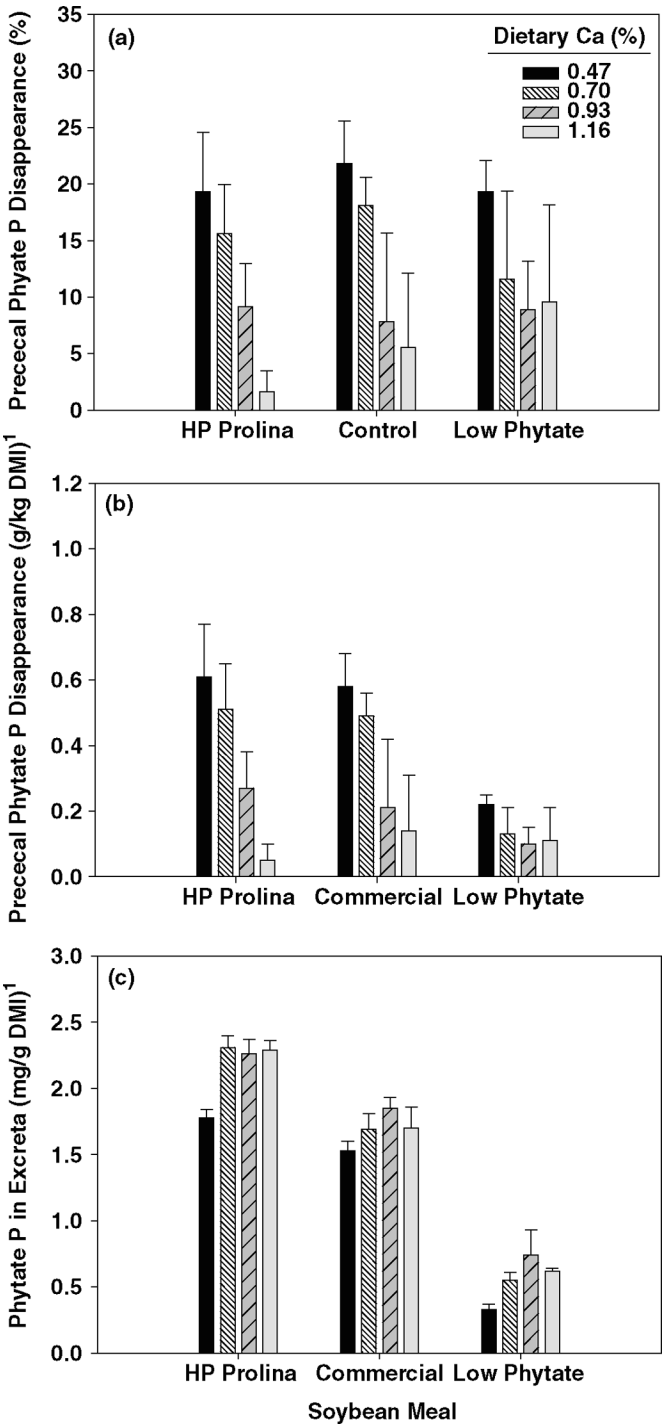

Figure 2. Effects of source of soybean meal with different phytate concentrations and dietary Ca on apparent prececal phytate P disappearance (a,b) of broilers at 21 d of age or phytate P in excreta from collection II (19 to 20 d; c). HP = high phytate. [1]Prececal phytate P disappearance and phytate P in excreta expressed in DM intake (DMI). Columns represent the average response obtained from 4 replicate pens of 10 broilers (a,b) or 13 broilers (c).

IP esters. The concentration of IP esters was lower in the LP SBM than the other SBM sources with the exception of IP3. The HP Prolina SBM had a greater concentration of IP5, which accounted for ~19% of the total IP content, whereas there was only ~11% IP5 in both the commercial and LP SBM.

The calculated and analyzed nutrient content of diets are presented in Table 3. Analyzed dietary CP, amino acids, Ca, and P levels were in good agreement with formulated values, and small deviations in the analyzed Ca and P values were attributed to sampling and analytical error. The analyzed phytate P concentration of sam-

Table 4. Main effect means[1] of source of soybean meal (SBM) and dietary Ca level on apparent prececal digestibility, absorption, and output of P, Ca, and phytate P at the distal ileum of broilers at 21 d of age

	Apparent prececal digestibility			Apparent prececal absorption[2]			Output at the distal ileum[2]		
Main effect	P	Ca	Phytate P	P	Ca	Phytate P	P	Ca	Phytate P
SBM	%			g/kg of DMI					
High-phytate Prolina	51.69^{b}	39.62	11.43	3.77^{a}	3.56	0.36^{a}	3.52^{b}	5.58	2.72^{a}
Commercial	52.54^{b}	40.68	13.32	3.14^{b}	3.82	0.35^{a}	2.84^{b}	5.20	2.29^{b}
Low phytate	65.44^{a}	42.96	12.33	3.05^{b}	3.53	0.14^{b}	1.61^{c}	5.50	0.97^{c}
SEM	1.15	3.24	3.49	0.07	0.34	0.08	0.07	0.34	0.08
Diet Ca, %									
0.47	64.32^{a}	46.76	20.15^{a}	3.78^{a}	2.44^{c}	0.47^{a}	2.19^{c}	2.78^{d}	1.84^{b}
0.70	57.85^{b}	40.59	15.09^{ab}	3.40^{b}	3.16^{bc}	0.37^{ab}	2.57^{b}	4.62^{c}	1.97^{ab}
0.93	53.55^{c}	38.41	8.61^{b}	3.14^{c}	3.97^{ab}	0.19^{bc}	2.84^{a}	6.37^{b}	2.03^{ab}
1.16	50.50^{c}	38.58	5.87^{b}	2.95^{c}	4.98^{a}	0.10^{c}	3.02^{b}	7.92^{a}	2.14^{a}
SEM	1.24	3.50	3.75	0.08	0.37	0.08	0.08	0.37	0.08
Source of variation	Probability > *F*								
SBM	<0.001	0.571	0.854	<0.001	0.803	0.002	<0.001	0.803	<0.001
Ca	<0.001	0.101	0.003	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SBM × Ca	0.800	0.571	0.888	0.009	0.696	0.034	0.009	0.711	0.421
Ca (linear)	<0.001	0.029	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Ca (quadratic)	0.069	0.232	0.713	0.067	0.630	0.975	0.067	0.630	0.787

[a–c]Means within the same column with no common superscript differ significantly ($P \leq 0.05$).

[1]Means represent the average response of 16 pens of 10 broilers each within each SBM source or 12 pens of 10 broilers within each Ca level.

[2]Expressed in grams per kilogram of DM intake (DMI).

ples of SBM (Table 1) was determined from samples of SBM drawn at the time of diet mixing and was lower compared with values used in diet formulation, which had been obtained from initial screening of HP Prolina SBM several months before the onset of the study. Therefore, although all diets had been formulated to contain the same calculated NPP of 0.35% by variable addition of feed-grade monobasic Ca phosphate ($CaH_4[PO_4]_2 \cdot H_2O$), differences between formulated and analyzed phytate P levels of the diets resulted in the analyzed diet NPP values of the HP Prolina diets being higher (0.40%) than the commercial and LP SBM diets (0.31 and 0.32%, respectively).

Broilers appeared healthy throughout the 5-d experimental period with the exception of 1 bird on the HP Prolina SBM diet with 0.70% Ca that died on the second day after the onset of the feeding study. The feed intake of chicks over the 5-d experimental period was similar between sources of SBM and was 422 ± 4.3 g for the HP Prolina diets, 424 ± 4.7 g for the commercial SBM diets, and 424 ± 3.2 g for birds consuming the LP SBM diets. The BW selection process at 12 d and similar feed intake of chicks on different treatments produced no differences in the 21-d BW of the 10 chicks that were randomly sampled per pen for collection of the ileal digesta. The 21-d BW was 864 ± 6.6, 856 ± 4.8, and 856 ± 4.8 g for the chicks from the HP Prolina, commercial, and LP SBM diets, respectively.

Apparent Prececal Digestibility and Output of P, Ca, and Phytate P at the Distal Ileum

The apparent prececal digestibility of P decreased in a curvilinear manner with increasing dietary Ca and was significantly higher for diets containing the LP SBM compared with either commercial or HP SBM (Table 4, Figure 1a). The apparent prececal P absorption per kilogram of DMI was not different between the commercial and LP SBM diets (Table 4, Figure 1b) but as a result of the higher analyzed NPP levels was greater in diets that contained HP Prolina SBM compared with diets with commercial or LP SBM. Further, a significant interaction of dietary Ca level and SBM source suggested that increasing dietary Ca concentration reduced the amount of prececal P absorbed at the distal ileum from the HP Prolina SBM more rapidly than from commercial or LP SBM, for which the response in P absorption and P output per kilogram of DMI at the distal ileum was similar (Table 4, Figure 1b,c).

There were no differences among SBM for the percentage of apparent prececal disappearance of phytate P, which decreased linearly from 20.15 to 5.87% when dietary Ca was increased from 0.47 to 1.16% (Table 4). However, differences in the initial diet phytate P intake per kilogram of DMI resulted in more phytate P absorbed from HP Prolina and commercial SBM diets (Table 4, Figure 2a,b), whereas the output of intact phytate P in the excreta was lowest for the LP SBM (Table 5, Figure 2c). Increasing dietary Ca concentrations also increased the output of phytate P per kilogram of DMI at the distal ileum and was independent of the dietary phytate P concentration.

Although there were differences in dietary phytate P concentrations from different sources of SBM, there was no evidence of an effect of phytate level or interaction of phytate and dietary Ca level on the apparent prececal Ca digestibility or amount of Ca absorbed per kilogram of DMI (Table 4). The prececal Ca digestibility increased 2-fold when dietary Ca level was increased from 0.47 to 1.16% (Table 4). However, in spite of more Ca being absorbed, the increase in dietary Ca from 0.47 to 1.16% also resulted in a significant stepwise increase in the output

Table 5. Main effect means[1] of source of soybean meal (SBM) and dietary Ca level on retention or output of P, Ca, and phytate P in the excreta of broilers from collection I (16 to 17 d, no dietary adaptation) and collection II (19 to 20 d)

	Collection I		Collection II						
	Percentage retention		Percentage retention		Retention (per kg of DMI)[2]		Excretion (per kg of DMI)[2]		
Main effect	P	Ca	P	Ca	P	Ca	P	Ca	Phytate P
SBM	%		%		g/kg of DMI				
High-phytate Prolina	32.01^{c}	47.87^{b}	38.95^{c}	38.36^{b}	2.84^{b}	3.35^{b}	4.45^{a}	5.78^{a}	2.16^{a}
Commercial	39.37^{b}	47.10^{b}	46.89^{b}	39.83^{b}	2.80^{b}	3.39^{b}	3.17^{b}	5.62^{b}	1.69^{b}
Low phytate	53.78^{a}	59.40^{a}	63.37^{a}	50.22^{a}	2.95^{a}	4.35^{a}	1.70^{c}	4.67^{b}	0.56^{c}
SEM	0.80	0.81	0.49	1.25	0.03	0.12	0.03	0.12	0.065
Diet Ca, %									
0.47	37.08^{c}	48.80^{b}	43.40^{c}	50.80^{a}	2.48^{c}	2.65^{c}	3.49^{a}	2.57^{d}	1.21^{b}
0.70	41.83^{b}	53.26^{a}	49.42^{b}	45.29^{b}	2.83^{b}	3.52^{b}	3.15^{b}	4.26^{c}	1.52^{a}
0.93	42.80^{ab}	52.95^{a}	52.96^{a}	41.32^{b}	3.07^{a}	4.27^{a}	2.91^{c}	6.06^{b}	1.62^{a}
1.16	45.16^{a}	50.80^{ab}	53.17^{a}	33.81^{c}	3.08^{a}	4.36^{a}	2.89^{c}	8.54^{a}	1.54^{a}
SEM	0.88	0.92	0.57	1.42	0.03	0.14	0.03	0.14	0.070
Source of variation	Probability > *F*								
SBM	<0.001	<0.001	<0.001	<0.001	<0.001	0.799	<0.001	0.799	<0.001
Ca	<0.001	0.003	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SBM × Ca	0.363	0.004	<0.001	0.215	<0.001	0.126	<0.001	0.118	0.483
Ca (linear)	<0.001	0.159	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Ca (quadratic)	0.100	<0.001	<0.001	0.294	<0.001	0.007	<0.001	0.007	<0.001

[a–d]Means within the same column with no common superscript differ significantly ($P \leq 0.05$).

[1]Means represent the average response of 16 pens of 13 broilers within each SBM source or 12 pens of 13 broilers within each Ca level.

[2]Expressed in grams per kilogram of DM intake (DMI).

of Ca from the distal ileum from 2.78 g/kg to as high as 7.92 g/kg of DMI (Table 4).

Total Retention and Excretion of P, Ca, and Phytate P in the Excreta During the First 24 h (Collection I) or After a 3-d Adaptation Period (Collection II)

There was a strong negative correlation between the percentage of P retention and the analyzed dietary phytate P concentration during both excreta collection I (r = –0.916; $P < 0.0001$) and during collection II (r = –0.922; $P < 0.0001$). During excreta collection I, the percentage of dietary P that was retained increased from 32.01 to 39.37% when commercial SBM replaced HP Prolina SBM in diets and to 53.78% when LP SBM replaced HP Prolina SBM (Table 5). After the 3-d adaptation period, the percentage of P retained during excreta collection II was 38.95, 46.89, and 63.37% for the HP Prolina, commercial, and LP SBM, respectively.

Increasing the dietary Ca level resulted in a linear increase in P retention during excreta collection I (Table 5), whereas the response in P retention to increasing Ca levels during excreta collection II differed among sources of SBM, which could be described by a quadratic function that reached a definite plateau (Figure 3a,b). Segmented regression analysis of the response in P utilization (i.e., retention and excretion) with increasing dietary Ca determined the breakpoint in the dietary Ca at which the plateau in P utilization was reached, which was 0.996 ± 0.057% Ca for the HP Prolina SBM diets, 0.739 ± 0.038% Ca for the commercial SBM, and 0.743 ± 0.053% Ca for the LP SBM diets (Figure 3a,b,c). Total P excretion per kilogram of DMI during collection II generally reflected differences in P retention, and the significant interaction of SBM and Ca level suggested that the initial reduction in excreta P output per kilogram of DMI with increasing dietary Ca concentration differed among sources of SBM, being lower for diets that contained LP SBM (Figure 3c). There was no interaction of dietary Ca level and source of SBM for phytate P output per kilogram of DMI in the excreta from collection II, but phytate P was 4-fold lower when LP SBM, rather than HP Prolina SBM, was included in the diet.

DISCUSSION

Previous workers who have investigated effects of phytate P concentration on P utilization obtained a range of phytate P in their diets either by adding synthetic sources of Ca or Mg phytate to diets (Waldroup et al., 1963a), by including graded levels of SBM (Dilger and Adeola, 2006), by reducing dietary phytate intake by pretreating ingredients with phytase enzymes (Nelson et al., 1968b), or by including LP variants of SBM in diets (Sands et al., 2003; Dilger and Adeola, 2006). In the present study, graded levels of dietary phytate P from SBM that ranged from 0.10 to 0.28% were obtained by combining a single source of DGDH corn with a low level of phytate P with 3 sources of SBM that differed in their natural concentration of phytate P. Inclusion of DGDH corn has previously been shown to reduce phytate P in diets without negatively affecting the performance of broilers or swine (Moeser et al., 2002; Applegate, 2005). Therefore, because diets in the present study contained similar levels of DGDH corn and SBM, they were similar in physical attributes that resulted in similar feed intake. Therefore, differences in P utilization observed in this study could probably be attributed

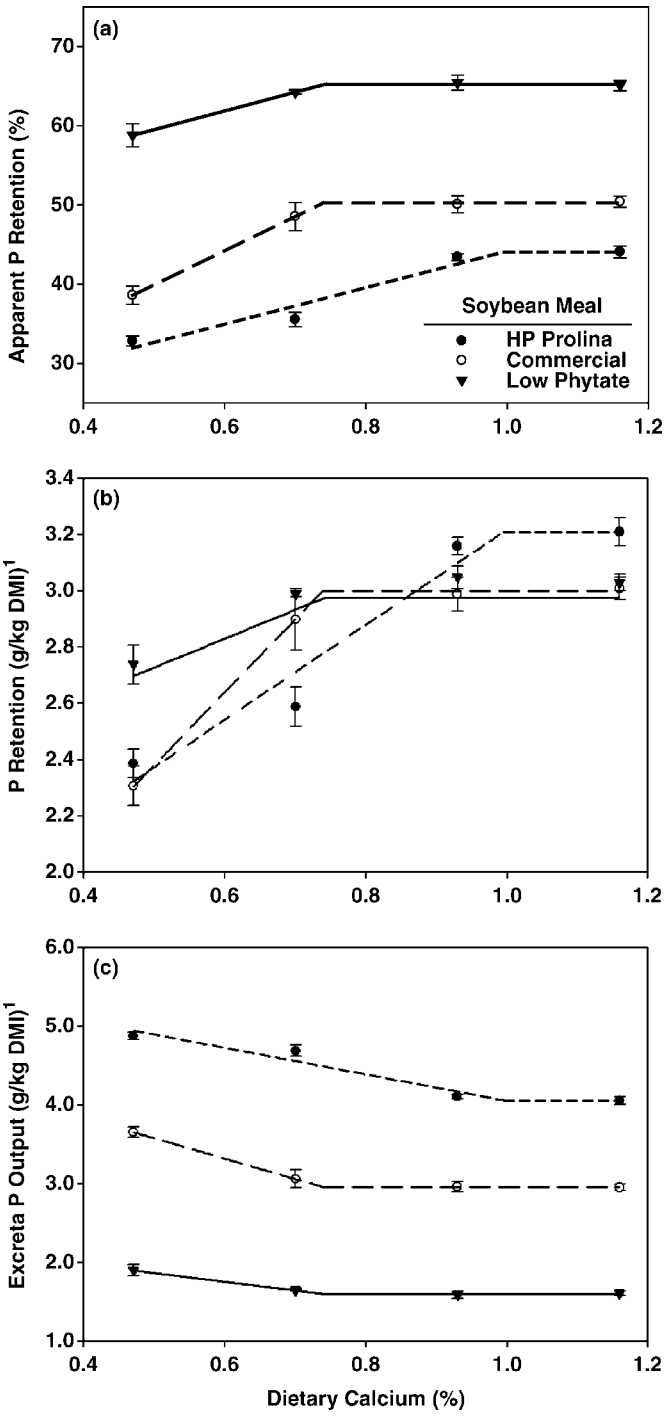

Figure 3. Effects of source of soybean meal with different phytate concentrations and dietary Ca on retention (a, b) and output (c) of P in the excreta of broilers from collection II (19 to 20 d). HP = high phytate. [1]Phosphorus retention and excreta P output expressed in grams per kilogram of DM intake (DMI). Symbols represent the average response obtained from 4 replicate pens of 13 broilers each.

to differences in the Ca, P, NPP, and phytate P content of diets rather than to differences in the physical attributes and consumption of the diets.

The higher determined NPP concentration of the HP Prolina SBM diets was unintentional and resulted from a lower-than-expected analyzed phytate concentration determined in a sample of SBM that was drawn when diets were mixed, compared with a previous sample drawn from the HP Prolina SBM 14 mo earlier. It was not known if the differences in analyzed phytate in the HP Prolina SBM were due to sampling error or if there had been some hydrolysis of the phytate to lower-order IP esters during storage.

Phytate P Disappearance and Apparent Digestibility of P at the Distal Ileum

The antagonism of dietary Ca on the apparent digestibility and absorption of P from the small intestine has been well established and was shown to be dependent on both the absolute Ca and P concentrations and the ratio of Ca to P in the diet (Hurwitz and Bar, 1971; Van der Klis and Versteegh, 1996). Several other studies have also found reduced P digestibility (Al-Masri, 1995), broiler performance, and bone mineralization (Waldroup et al., 1963b; Qian et al., 1997; Driver et al., 2005) when the Ca:P ratio was widened in diets with low levels of P. The negative effect of Ca on the apparent digestibility of P in the intestines has been thought to result from 2 potential mechanisms. In the first instance, elevated concentrations of dietary Ca were hypothesized to increase the formation of insoluble Ca-phytate complexes that reduced phytate P hydrolysis by endogenous or exogenous phytase enzymes (Nelson, 1980; Wise, 1983; Qian et al., 1997). Second, excess Ca relative to inorganic P increased the formation of inorganic Ca-P precipitates, which decreased the concentrations of soluble forms of P in the intestinal lumen and reduced P digestibility (Hurwitz and Bar, 1971). Given these 2 separate mechanisms whereby dietary Ca could potentially reduce the apparent digestibility of P, we hypothesized at the onset of this study that the response in apparent prececal P digestibility and overall P utilization (retention and excretion) to increasing dietary Ca concentration may be different when diets contained different concentrations of phytate.

There was no interaction of dietary Ca and phytate from SBM on apparent P digestibility, which improved from 51.69 and 52.54% to 65.44% when LP SBM was included in diets in place of either commercial SBM or HP Prolina, respectively (Table 4). The effects of Ca and phytate concentration on ileal P digestibility and absorption can be evaluated by comparing the response of the LP SBM and commercial SBM diets. The respective analyzed concentrations of NPP were 0.32 and 0.31%, whereas diets with LP SBM and commercial SBM had large differences in the concentration of phytate P (0.10 and 0.24%, respectively) and large differences in the levels of added monobasic Ca phosphate (0.59 or 1.09%, respectively). In spite of the substantial differences in the phytate P of the commercial and LP SBM diets, there was no difference in the rate at which prececal P digestibility or absorption was reduced when dietary Ca was increased from 0.47 to 1.16%. The observed reduction in P digestibility with increasing Ca was consistent with previous research (Van der Klis and Versteegh, 1996). Importantly, at the same analyzed NPP level, the inclusion of the LP SBM in place

of commercial SBM resulted in similar amounts of P absorbed per kilogram of DMI but decreased total P output in the excreta by 49%. This suggested that the inclusion of the LP SBM in diets was able to replace the additional 0.12% P from monobasic Ca phosphate that had been added to commercial SBM diets to maintain a similar level of dietary NPP.

The higher apparent P absorption per kilogram of DMI in diets containing the HP Prolina SBM and the significant interaction observed between Ca and SBM source for apparent P absorption per kilogram of DMI were most likely caused by the higher analyzed NPP concentration in the HP Prolina SBM that resulted in a more rapid decrease in apparent P absorption when dietary Ca concentrations were increased. The improved P digestibility and retention of P in diets containing LP SBM were consistent with previous studies that compared LP vs. conventional sources of SBM (Sands et al., 2003), corn (Li et al., 2000), and barley (Thacker et al., 2003). In contrast to these findings, Dilger and Adeola (2006) found no significant difference in the apparent prececal P digestibility of LP or conventional SBM, which was 85 and 82.6%, respectively. However, in the study by Dilger and Adeola (2006), diets contained no added Ca or P from inorganic mineral sources. Tamin et al. (2004) showed that in the absence of added Ca from $CaCO_3$ to diets, broilers were able to hydrolyze 69.2% of the dietary phytate P, which was reduced to 25.4% when 0.5% Ca from $CaCO_3$ was added to a corn-soy diet. Therefore, in the study of Dilger and Adeola (2006), the absence of added Ca and inorganic P sources would presumably have facilitated a high rate of phytate P hydrolysis, which may have ameliorated any differences in P digestibility between sources of SBM with low or high concentrations of phytate P.

In our study, the mean disappearance of phytate P at the distal ileum when diets contained 0.47% Ca was 20.15% (Table 4), which was lower than the previous estimates of Leske and Coon (1999) of 34.9% in diets with 0.5% Ca. The lower phytate P hydrolysis in our study was most likely due to the younger age of birds and the inclusion of higher levels of NPP, both of which have been shown to reduce the extent of phytate P utilization by broilers (Nelson, 1980; Van der Klis and Versteegh, 1996). Based on a lower percentage of phytate hydrolysis with increased dietary Ca, Wise (1983) suggested that the *M*-ratio of Ca:phytate in diets was one of the main factors determining the fate of phytate P. At a single level of dietary Ca, diets containing LP SBM in the present study would have had a *M*-ratio of Ca:phytate P in the intestine over 2-fold higher than diets containing the commercial or HP Prolina SBM. However, this increase in the Ca:phytate ratio did not affect the mean percentage of phytate P disappearance by the time digesta had reached the distal ileum. However, in agreement with previous findings (Nelson and Walker, 1963; Van der Klis and Versteegh, 1996; Tamin et al., 2004), a high dietary Ca concentration linearly decreased the percentage of disappearance of phytate P at the distal ileum, which would have contributed to the decrease from 64.32 to 50.50% in the apparent prececal digestibility of P at the highest level of dietary Ca. Therefore, these data suggested that the percentage of dietary phytate hydrolyzed in the small intestine may be affected to a greater extent by the concentration of Ca added to diets as $CaCO_3$ than by the ratio of Ca:phytate.

Effects of Ca:NPP Ratio and Phytate P Concentration on Overall P Retention

Although low levels of dietary Ca consistently increased phytate hydrolysis and the amount of P absorbed from the intestines, overall P retention responded positively to increasing dietary Ca concentrations and reached a plateau after which no further improvements occurred. This was consistent with previous observations by Van der Klis and Versteegh (1996), who showed that increasing the dietary Ca concentration reduced the digestibility of P at the ileum but increased the overall retention of P. These opposing effects of Ca on P digestibility vs. P retention at low dietary Ca levels could be attributed to an imbalance in the Ca:P ratio that was absorbed by the terminal ileum. Therefore, although lower dietary Ca levels increased phytate hydrolysis and P absorption in the intestines, the concurrent increase in P excretion suggested that there was insufficient Ca to allow the incorporation of the P into bone. Because the kidney plays an important role in the regulation of Ca and P levels in the plasma (Al-Masri, 1995), any excess circulating P would have been excreted in the urine, which was reflected in the higher total P in the excreta and reduced P retention at low dietary Ca concentrations.

Segmented regression analyses estimated the optimum Ca concentration at which P retention was maximized to be 0.74% Ca when diets contained commercial SBM (0.31% NPP) or LP SBM (0.32% NPP). Maximum P retention was reached in the HP Prolina SBM diet at 1.00% dietary Ca, but mean analyzed NPP levels of these diets were also higher (0.40% NPP). Therefore, the Ca:NPP ratio at which P retention was maximized was approximately 2.53:1 for the HP Prolina SBM diets, 2.40:1 for the commercial SBM diets, and 2.34:1 for the LP SBM diets.

The Ca:NPP ratio for maximum P retention and minimal P excretion estimated in the present study was similar to the optimal Ca:available P ratio of 2.2:1 and 2.3:1 estimated by Van der Klis and Versteegh (1996) for LP and HP diets, respectively. Also, a Ca:NPP ratio of 2.22:1 can be calculated from the NRC (1994) estimate of the Ca and NPP requirements of 1.00 and 0.45%, respectively, for 0- to 3-wk-old broilers.

The absence of any clear effect of dietary phytate concentration on Ca digestibility and retention was in contrast to earlier observations that the Ca requirement of 3-wk-old chicks was increased by at least 50% when dietary phytate levels were increased from 0.0 to 1.25% (Nelson et al., 1968b). The increased Ca requirement demonstrated in the study by Nelson et al. (1968b) was hypothesized to be caused by increased binding of Ca in the intestine, because 1% phytate was found to be able to bind 0.36% Ca. The differences in the effects of phytate on Ca digestibility

observed in the present study may be attributed to lower phytate concentrations from SBM. The large range in phytate of 1.25% in the study by Nelson et al. (1968b) was obtained by adding sodium phytate to semisynthetic diets, which may have contributed to the reported effects of phytate on the dietary Ca requirement.

In conclusion, the present study suggested that although including LP SBM significantly increased ileal digestibility of P, there was no effect of phytate level on Ca digestibility. Ileal P digestibility decreased when dietary Ca increased from 0.47 to 1.16%. In contrast, a minimum level of dietary Ca was required for retention of P that had been absorbed in the intestines. The optimum ratio of Ca:NPP at which P retention was maximized was dependent upon the dietary phytate level, increasing from 2.3:1 to 2.5:1 when phytate P increased from 0.10 to 0.28%. Further research may be needed to ascertain the effects of such a wide Ca:P ratio on long-term broiler performance and economic returns.

ACKNOWLEDGMENTS

This work was funded in part by the United Soybean Board and the North Carolina Agricultural Foundation.

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