

Effects of Low Phytic Acid Corn on Phosphorus Utilization, Performance, and Bone Mineralization in Broiler Chicks¹

Y. C. Li,* D. R. Ledoux,*² T. L. Veum,* V. Raboy,† and D. S. Ertl‡

*Department of Animal Sciences, University of Missouri, Columbia, Missouri 65211; †USDA, Agricultural Research Service, National Small Grain Germplasm Research Facility, Aberdeen, Idaho 83210; and ‡Pioneer Hi-Bred International, Johnston, Iowa 50131

ABSTRACT In vivo and in vitro experiments were conducted to determine whether P in a low-phytate corn (LPC) containing the *lpa 1-1* allele is more available than P in a near-isogenic wild-type corn hybrid (NC). The LPC was analyzed to contain 0.18% nonphytate P and 0.26% total P (TP), whereas NC contained 0.05% nonphytate P and 0.25% TP. For these studies, nonphytate P was considered to be available P (AP). In the in vivo study, 150 1-d-old male chicks were randomly assigned to five treatments (six pens of five chicks each) for 21 d. The dietary treatments included: A) a diet containing 60% NC, 0.2% AP, and 0.8% Ca; B) a diet containing 60% LPC, 0.28% AP, and 0.8% Ca; C) an NC diet similar to Diet A, but with KH₂PO₄ added to increase the AP to 0.28% to match the AP in Diet B; D) an LPC diet containing 0.45% AP and 1% Ca; and E) an NC diet supplemented with KH₂PO₄ to provide 0.45% AP and 1% Ca. Diets A, B, and C were semipurified diets, with corn being the sole source of phytate. The only differences between Diets A and B were the source of corn and the amount of AP present in the diets. The levels of AP in these diets were deficient

in order to measure the animal response to the different levels of AP. Diets D and E were typical corn-soybean meal diets, and were formulated to contain an optimal level of AP. Performance and bone ash were similar ($P > 0.05$) in chicks fed Diets B and C and in chicks fed Diets D and E. Chicks fed LPC diets (B and D) retained more P ($P < 0.05$) than chicks fed NC diets (C and E). Chicks fed Diet B had significantly higher ($P < 0.05$) Ca retention compared with chicks fed Diet A. An in vitro digestion procedure that simulated the physiological conditions of the gastrointestinal tract of broilers was used to determine P release from LPC and NC. Results showed that 65% (1,420 mg/kg) of the TP in LPC was released, compared with 23% (543 mg/kg) from NC. Results of these experiments indicate that the P in LPC is more available than the P in NC, and reducing the phytate content did not compromise the nutritional value of LPC. The increased P retention in chicks fed LPC suggests that substituting LPC for NC leads to a reduction in manure P. Also, the in vitro procedure accurately predicted differences in in vivo P availability between the two corns.

(Key words: chicks, low-phytate corn, phosphorus)

2000 Poultry Science 79:1444–1450

INTRODUCTION

With few exceptions, poultry rations are based largely on cereal grains and oilseed meals. Unfortunately, approximately two thirds of the P in cereal grains and oilseed meals is present in the form of P bound to phytic acid (phytate P), which is not digested by poultry; most is excreted in the manure. As a result, approximately 250,000 tons of manure P is produced annually and contributes to water pollution (Cromwell, 1994). Poultry manure is often used as fertilizer on pastures and other

croplands. In areas of intensive poultry production, however, the nutrient content of manure often exceeds the requirements for plant growth. If this excess occurs, the additional P can contribute to a significant environmental problem.

A number of nutritional approaches are currently being evaluated to deal with the poor availability of phytate P and the resultant potential for P pollution. These approaches include: 1) formulating diets at the requirements of poultry to avoid excess P excretion; 2) adding microbial phytase to poultry diets to increase phytate P availability; and 3) genetically lowering the phytic acid content of cereal grains and oilseeds, thereby improving plant P availability. Recently, USDA scientists devel-

Received for publication February 8, 2000.

Accepted for publication June 19, 2000.

¹Contribution Number 12940 of the Missouri Agricultural Experiment Station.

²To whom correspondence should be addressed: ledoux@missouri.edu.

Abbreviation Key: AP = available phosphorus; LPC = low-phytate corn; NC = wild-type corn; TP = total phosphorus.

oped two nonlethal low-phytate corn (LPC; *Zea mays* L) types, *lpa1-1* and *lpa2-1*, which are phenotypically identical to wild-type corn (NC) hybrids. These LPC show 60% (*lpa1-1*) and 50% (*lpa2-1*) reductions in phytic acid P, with no reduction in total P in the seed (Ertl et al., 1998). Chick studies have demonstrated that the relative bioavailability of P in LPC (*lpa1-1*) ranged from 45 to 52%, compared with 10% for NC (Cromwell et al., 1998). Phosphorus excretion was also significantly reduced in chicks fed the LPC (Cromwell et al., 1998). The objectives of the present *in vivo* study were to confirm the increased bioavailability of P in the LPC observed in previous studies, and also to determine whether the decreased phytate content in LPC would change the nutritional value of LPC. In addition, an *in vitro* procedure that simulates the physiological conditions in different parts of the broiler gastrointestinal tract was evaluated to determine whether it could be used to detect differences in P availability from NC and LPC.

MATERIALS AND METHODS

In Vivo Study

One-hundred-fifty 1-d-old male chicks were purchased from a commercial hatchery,³ weighed, wing-banded, and randomly assigned to treatments. A completely randomized design was used with five dietary treatments and six replicate pens of five chicks allotted randomly to each dietary treatment. Dietary treatments (Table 1) included: A) a diet containing 60% NC, 0.2% available P (AP), and 0.8% Ca; B) a diet containing 60% LPC, 0.28% AP, and 0.8% Ca; C) an NC diet similar to diet A, but with KH_2PO_4 added to increase the AP to 0.28% in order to match the AP in diet B; D) an LPC diet containing 0.45% AP, 0.62% total P (TP), and 1% Ca; and E) an NC diet supplemented with KH_2PO_4 to provide 0.45% AP, 0.69% TP, and 1% Ca.

Diets A, B, and C were semipurified-type diets containing mainly glucose, casein, and gelatin. Corn was the only source of phytate in these diets. The reason that semipurified diets were used in the current study was to determine whether lower phytate content in LPC would affect the nutritional value of LPC. The levels of AP in these diets were below the bird's requirement in order to measure the bird's response to different levels of AP (NRC, 1994). Diets A and B were designed to determine whether P in LPC is more available than P in NC. Because the only difference between Diets A and B was the source of corn, differences observed in any response variables should be a result of different AP levels in the diets. Diet C was Diet A supplemented with inorganic P to

increase the AP level in order to match the AP in the Diet B, so that the only difference in these two diets was the level of phytic acid. Diet C was then compared with Diet B to determine whether the decreased phytate P content in LPC would affect the nutritional value of LPC. In addition, because Diets A, B, and C were semipurified diets and contained levels of AP lower than the NRC requirement (NRC, 1994), Diets D and E, which were commercial-type (corn-soybean meal) diets, were also included in the current study. These two diets were formulated to contain optimal levels of AP, with the LPC providing a greater proportion of AP (Diet D) compared with that of NC (Diet E).

Both the LPC and the NC used in the current study were provided by Pioneer Hi-Bred International.⁴ The LPC was homozygous for the low phytic acid 1-1 (*lpa1-1*) allele of the low phytic acid gene (Ertl et al., 1998), and was analyzed to contain 0.26% TP and 0.18% nonphytate P, whereas NC was a near-isogenic, wild-type corn hybrid, and was analyzed to contain 0.25% TP and 0.05% nonphytate P (Raboy et al., 1984, 1990). In this study, nonphytate P was considered as AP. Samples of both NC and LPC were analyzed for nutrient contents and amino acid profiles (Table 2; AOAC, 1990).⁵ The composition and selected nutrient composition of experimental diets are given in Table 1. With the exception of Ca and P in diets A, B, and C, all diets were formulated to meet the nutrient requirements of broilers (NRC, 1994). Chromic oxide (CrO_3 ; 0.05%) was included in the diets as an indigestible marker for the determination of P and Ca retention.

Chicks were placed on experimental diets from Day 1 to Day 21, and were housed in stainless steel battery brooders with wire-mesh floors, with temperature kept at 37 C at the first week of the experiment. The temperature was decreased by approximately 2 C every 7 d. Chicks were maintained on a 24-h constant-light schedule and allowed access to feed and water *ad libitum*. Chicks were weighed individually, and feed consumption was determined for each pen on a weekly basis. During the third week, excreta samples were collected for 5 consecutive d for determination of P and Ca retention. On Day 21, chicks were killed by asphyxiation with CO_2 , and middle toes from both feet were collected from each chick for determination of toe ash. Toes were dried at 100 C for 24 h and ashed in a muffle furnace at 600 C overnight. The right tibiae (three chicks per pen) were also collected for determination of tibia ash. Tibiae were stripped of adhering tissue and dried at 100 C, and fat was extracted with a mixture of ethanol and methanol (90 and 10%, respectively). Fat-extracted tibiae were then dried at 100 C for 24 h and ashed in a muffle furnace at 600 C overnight. Excreta were dried in an oven at 50 C for 24 h and ground to pass through a 1-mm sieve. Feed samples were also ground to pass through a 1-mm sieve. Duplicate samples of excreta and feed were digested by nitric-perchloric acid wet digestion, and the assay was validated by including standard reference material (peach leaves) from the National Institute of Standards

³Stover Hatchery, Stover, MO 65078.

⁴Pioneer Hi-Bred International, Inc., Johnston, IA 50131.

⁵Experiment Station Chemical Laboratories, University of Missouri, Columbia, MO 65211.

TABLE 1. Composition and selected nutrient content of experimental diets

Item	Diet				
	A	B	C	D	E
	(%)				
Ingredients					
Wild-type corn	60	...	60	...	57.8
Low-phytate corn	...	60	...	57.8	...
Soybean meal (48%)	30.6	30.6
Glucose	13.8	13.8	13.8
Casein	9.1	9.1	9.1
Gelatin	7.0	7.0	7.0
Fish meal	5.9	5.9	5.9	6.0	6.0
Corn oil	2.8	2.8
L-lysine	1.1	1.1	1.1
Limestone	1.0	1.0	1.0	1.2	1.2
Dicalcium phosphate	0.6	0.6
Salt	0.6	0.6	0.6	0.4	0.4
DL-methionine	0.5	0.5	0.5	0.14	0.14
KCL	0.2	0.2	0.2
L-tryptophan	0.14	0.14	0.14
Choline	0.12	0.12	0.12
Trace mineral mix ¹	0.11	0.11	0.11	0.11	0.11
Vitamin mix ²	0.05	0.05	0.05	0.05	0.05
Selenium mix ³	0.05	0.05	0.05	0.05	0.05
Chromic oxide	0.05	0.05	0.05	0.05	0.05
Cellufil	0.35	0.35	...	0.33	...
KH ₂ PO ₄	0.35	...	0.33
Nutrient contents					
ME, kcal/kg	3,200	3,200	3,200	3,200	3,200
CP, %	23	23	23	23	23
Ca, %	0.8	0.8	0.8	1.0	1.0
Calculated total P, %	0.41	0.42	0.49	0.62	0.69
Analyzed total P, %	0.43	0.42	0.52	0.64	0.74
Estimated available P, %	0.20	0.28	0.28	0.45	0.45

¹Mineral mix provided in milligrams per kilogram of diet: MnO₂, 222; ZnO, 209; FeSO₄·7H₂O, 654; CuSO₄, 32 mg; ethylenediamine dihydroiodide, 1.9 mg; CaCO₃, 160.

²Supplied per kilogram of feed: vitamin A (retinyl acetate), 8,810 IU; cholecalciferol, 3,855 IU; vitamin E (dl- α -tocopheryl acetate), 14 IU; niacin, 55 mg; calcium pantothenate, 17 mg; riboflavin, 6.6 mg; pyridoxine, 2.2 mg; menadione sodium bisulfite, 1.7 mg; folic acid, 1.4 mg; thiamin mononitrate, 1.1 mg; biotin, 0.2 mg; cyanocobalamin, 11 μ g; ethoxyquin, 83 mg.

³Supplied 0.3 mg selenium per kilogram of feed.

and Technology.⁶ Phosphorus concentrations in feed were determined colorimetrically by the molybdo-vanadate method (AOAC, 1970). Calcium and Cr were analyzed by flame atomic absorption spectrophotometry. Phosphorus and Ca retention were calculated using the following formula: $100\% - [100\% \times (\text{Cr concentration in feed} \div \text{Cr concentration in feces}) \times (\text{P or Ca concentration in feces} \div \text{P or Ca concentration in feed})]$. The animal care and use protocol was reviewed and approved by the University of Missouri-Columbia Animal Care and Use Committee.

In Vitro Phosphorus Release Study

Both NC and LPC samples were ground through a 1-mm sieve and refrigerated until analysis. The in vitro

digestion procedure of Zyla et al. (1995) was used in this study with some modifications. Briefly, approximately 1 g of corn sample was weighed into a 50-ml centrifuge tube.⁷ Each sample was hydrated with 2 ml of 0.03 M HCl, and the tube was vortexed to facilitate adequate mixing of the sample with HCl solution. Samples were incubated in a water bath at 40 C for 30 min. At the end of the incubation, 0.5 ml of 1.5 M HCl containing 3,000 U pepsin⁸ was added to each tube, mixed well, and incubated for 45 min at the same temperature. At the end of this incubation period, 0.65 ml of 1 M NaHCO₃ containing 3.7 mg pancreatin/ml (8 \times United States Pharmacopeia)⁸ was slowly added to each sample and mixed well, and samples were incubated for 2 h at the same temperature. At the end of the incubation period, samples were centrifuged at 2,500 rpm for 15 min, and the supernatant from each sample was collected for P determination. The in vitro procedure was replicated six times for evaluation of P release from NC and LPC. Phosphorus concentration in the supernatant was determined colorimetrically by the molybdo-vanadate method (AOAC, 1970).

⁶U.S. Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD 20899.

⁷Fisher Scientific, Pittsburgh, PA 15219.

⁸Sigma Chemical Co., St. Louis, MO 63178-9916.

Statistical Analyses

All data were analyzed by analysis of variance using the general linear models procedure of SAS® software (SAS Institute Inc., 1996). Means for treatments showing significant differences in the analysis of variance were compared by Fisher's protected least significant difference procedure (Snedecor and Cochran, 1967). In the *in vivo* study, pen was used as the experimental unit. Dietary treatments were compared by single degree of freedom contrasts. For the *in vitro* study, Student's *t*-test was employed to analyze the data (Snedecor and Cochran, 1967). Statistical significance was accepted at $P < 0.05$.

RESULTS

In Vivo Study

The effects of dietary treatments on growth performance are presented in Table 3. Compared with chicks fed the semipurified diet containing NC (Diet A), chicks fed the semipurified diet containing LPC (Diet B) consumed 17% more feed, gained 24% more weight, and were more efficient (1.37 vs. 1.45, g:g) in converting feed to gain ($P < 0.05$). Chicks fed Diet B had similar ($P > 0.05$) feed intake, weight gain, and feed efficiency to chicks fed the semipurified diet containing NC plus KH_2PO_4 (Diet C). Results also showed that feed intake, body weight gain, and feed conversion of chicks fed the corn-soybean meal diet containing LPC (Diet D) were not significantly different ($P > 0.05$) from those fed the corn-soybean meal diet containing NC plus KH_2PO_4 (Diet E).

The effects of dietary treatments on bone mineralization are presented in Table 4. Compared with chicks

fed Diet A, chicks fed Diet B had significantly higher percentage tibia and toe ash ($P < 0.05$). Chicks fed Diet B had significantly higher percentage tibia ash ($P < 0.05$) compared with chicks fed Diet C. Chicks fed Diet B had similar toe ash to chicks fed Diet C ($P > 0.05$). There was no significant difference in percentage tibia or toe ash between chicks fed Diets D and E ($P > 0.05$). The effects of dietary treatments on P and Ca retention are presented in Table 5. Chicks fed Diet B had significantly higher P retention compared with chicks fed Diet A ($P < 0.05$). Chicks fed Diet B also had significantly higher P retention compared with chicks fed Diet C ($P < 0.05$). Compared with chicks fed Diet E, P retention of chicks fed Diet D was significantly higher ($P < 0.05$). Chicks fed Diets B and C had significantly higher Ca retention compared with chicks fed Diet A ($P < 0.05$). Calcium retention in chicks fed Diet D was not significantly different from that of chicks fed Diet E ($P > 0.05$). Chicks fed Diets A, B, or C retained more Ca than chicks fed Diets D and E.

In Vitro Phosphorus Release Study

In vitro P release from NC and LPC is presented in Table 6. Results showed that P release from LPC was significantly higher than the P release from NC ($P < 0.05$), with 23% of the P in NC being released, compared with 65% of the P in LPC.

DISCUSSION

As mentioned in the previous section, one of the objectives of the current study was to determine whether P in LPC was more available than P in NC. In the *in vivo* study, Diets A, B, and C were designed to address this objective. Results show that growth performance and bone mineralization of chicks fed the semipurified diet containing LPC (Diet B) were improved compared with chicks fed the semipurified diet containing NC (Diet A). The only differences between these two diets were the source of corn and the increased amount of AP present in Diet B due to the use of LPC (A: 0.2% AP; B: 0.28% AP). The better performance and bone mineralization observed in chicks fed Diet B suggest that P in LPC is more available than P in NC. In addition, a semipurified diet containing NC supplemented with KH_2PO_4 (Diet C) was formulated to contain 0.28% AP in order to match the level of AP in Diet B. Results showed that chicks fed Diets B and C had similar performance and bone mineralization. These data indicate that the estimated value of AP in LPC is reasonable, and that lowering the phytate content in LPC did not result in any detectable differences in the nutritional value of LPC.

Because Diets A, B, and C were semi-purified diets, and the AP level in these diets was lower than the NRC requirement, Diets D and E were formulated to contain the optimal level of AP in a practical corn-soybean meal-type diet to determine whether substituting LPC in commercial-type diets would affect chick performance. Results show that chicks fed the corn-soybean meal diet

TABLE 2. Analyzed nutrient content of wild-type and low-phytate corn¹

Item	Wild-type corn	Low-phytate corn
	(%)	
Crude protein	8.84	8.39
Crude fat	3.35	3.29
Crude fiber	1.41	1.23
Ash	1.18	1.19
Moisture	11.82	11.81
Arginine	0.38	0.39
Cysteine	0.19	0.20
Isoleucine	0.34	0.32
Leucine	1.19	1.08
Lysine	0.24	0.26
Methionine	0.15	0.16
Phenylalanine	0.47	0.43
Threonine	0.29	0.29
Tryptophan	0.06	0.06
Tyrosine	0.26	0.25
Valine	0.44	0.43
Analyzed P and Ca content		
Calcium	0.005	0.005
Phytate P	0.21	0.08
Nonphytate P	0.05	0.18
Total P	0.25	0.26

¹Values reported are on an as-fed basis.

TABLE 3. Performance of chicks fed diets containing wild-type and low-phytate corn¹

Item	Corn source ²	AP ³	TP ³	Feed intake	Weight gain	Feed:gain
		(%)	(%)	(g)	(g)	(g:g)
Diet ⁴						
A	NC	0.20	0.41	704 ^c	487 ^c	1.45 ^a
B	LPC	0.28	0.42	822 ^b	604 ^b	1.37 ^b
C	NC	0.28	0.49	775 ^b	574 ^b	1.35 ^b
D	LPC	0.45	0.62	1,008 ^a	807 ^a	1.25 ^c
E	NC	0.45	0.69	1,035 ^a	792 ^a	1.31 ^{bc}
Pooled SEM				24	19	0.001

^{a-c}Values within columns with no common superscripts differ significantly ($P < 0.05$).

¹Each value represents the mean of six pens of five chicks each.

²NC = wild-type corn; LPC = low-phytate corn.

³AP = available P, TP = total P; values in AP and TP columns are estimated values.

⁴A: an NC diet containing 0.2% AP and 0.8% Ca; B: an LPC diet containing 0.28% AP and 0.8% Ca; C: an NC diet plus 0.35% KH₂PO₄ to provide 0.2% AP and 0.8% Ca; D: a corn-soybean meal-type diet containing LPC, 0.45% AP, and 1% Ca; E: a corn-soybean meal-type diet containing NC plus KH₂PO₄ to provide 0.45% AP and 1% Ca.

containing LPC (Diet D) had similar performance and bone mineralization compared with chicks fed the corn-soybean meal diet containing NC plus KH₂PO₄ (Diet E), indicating that LPC had the same nutritional value as NC, and that replacing NC with LPC in the diet did not affect chick performance. Results of the current study confirm the increased P availability in LPC observed in previous studies (Cromwell et al., 1998; Ertl et al., 1998). Cromwell et al. (1998) reported that P in LPC is about four times as bioavailable as the P in NC for chicks (45% for LPC; 10% for NC). Based on the results of Ertl et al. (1998), P in LPC and NC was 74 and 43% available, respectively, compared with the standard, monosodium phosphate.

Results of the current study also indicate that chicks fed diets containing LPC had significantly higher P retention compared with chicks fed diets containing NC.

TABLE 4. Tibia and toe ash of chicks fed diets containing wild-type and low-phytate corn

Item	Corn source ¹	AP ²	TP ²	Tibia ash ³	Toe ash ⁴
		(%)	(%)	(%)	(%)
Diet ⁵					
A	NC	0.20	0.41	40.8 ^c	11.2 ^b
B	LPC	0.28	0.42	45.1 ^b	12.8 ^a
C	NC	0.28	0.49	41.7 ^c	12.9 ^a
D	LPC	0.45	0.62	47.4 ^a	12.8 ^a
E	NC	0.45	0.69	48.0 ^a	12.8 ^a
Pooled SEM				0.6	0.3

^{a-c}Values within columns with no common superscripts differ significantly ($P < 0.05$).

¹NC = wild-type corn; LPC = low-phytate corn.

²AP = available protein; TP = available protein; values in AP and TP columns are estimated values.

³Each value represents the mean of six pens of three chicks each.

⁴Each value represents the mean of six pens of five chicks each.

⁵A: an NC diet containing 0.2% AP and 0.8% Ca; B: an LPC diet containing 0.28% AP and 0.8% Ca; C: an NC diet plus 0.35% KH₂PO₄ to provide 0.2% AP and 0.8% Ca; D: a corn-soybean meal-type diet containing LPC, 0.45% AP, and 1% Ca; E: a corn-soybean meal-type diet containing NC plus KH₂PO₄ to provide 0.45% AP and 1% Ca.

Chicks fed Diet B, which contained LPC, retained 9.6 and 15.3% more P compared with chicks fed Diets A and C, which contained NC, respectively, which, again suggests higher P availability in LPC compared with NC. In addition, when LPC was substituted for NC in a commercial-type (corn-soybean meal) diet, P retention was increased by 6.7%. Results of the current study also showed that fecal P concentration was decreased by 52% in chicks fed LPC compared with chicks fed NC when corn was the sole source of phytic acid in the diet (LPC fecal P: 0.42%; NC fecal P: 0.81%). When LPC was substituted for NC in a commercial-type (corn-soybean meal) diet, there was a 22% reduction in P excretion (LPC fecal P: 1.34%; NC fecal P: 1.73%). It was observed that when P level in the diet was reduced, the effect of substituting LPC for NC on fecal P became more evident. It is possible that when P is limiting, more P is retained in the body for maintaining physiological functions, thus resulting in less P being excreted in the waste. This pattern was also observed in other species. In swine, Veum et al. (1999) reported that when corn was the sole source of phytic acid in the diet and AP concentration in the diet was limited, P excretion was reduced by 50% in growing pigs by substituting LPC for NC. When the optimum level of AP was provided in the diets, only a 22% reduction in P excretion was observed. In fish, Sugiura et al. (1999) observed a 41% reduction in total P excretion with replacement of commercial dent corn with LPC in the fish diet. Sugiura et al. (1999) also suggested that the difference in P availability between LPC and NC should become more evident by reducing the P concentration in the diet to the minimum levels required by fish.

Results of the current study also indicated that LPC increased Ca utilization. When corn was the sole source of phytic acid in the diets, Ca retention was increased by 21% by replacing NC with LPC (Diet A vs. Diet B). Similar findings were also observed in swine and fish (Sugiura et al., 1999; Veum et al., 1999), when NC was replaced by LPC in swine and fish diets. However, the current study also showed that chicks fed NC supple-

TABLE 5. Phosphorus and calcium retention of chicks fed diets containing wild-type and low-phytate corn

Item	Corn source ¹	AP ²	TP ²	Ca	P retention ^{3,4}	Ca retention ^{3,5}
		(%)	(%)	(%)	(%)	(%)
Diet ⁶						
A	NC	0.20	0.41	0.8	73.3 ^b	54.0 ^b
B	LPC	0.28	0.42	0.8	82.9 ^a	68.6 ^a
C	NC	0.28	0.49	0.8	67.6 ^c	74.0 ^a
D	LPC	0.45	0.62	1.2	51.3 ^d	33.3 ^c
E	NC	0.45	0.69	1.2	44.6 ^e	33.1 ^c
Pooled SEM					1.5	2.6

^{a-e}Values within columns with no common superscripts differ significantly (*P* < 0.05).

¹NC = wild-type corn; LPC = low-phytate corn.

²AP = available P; TP = total P; values in AP and TP columns are estimated values.

³Each value represents the mean of six pens of five chicks each.

⁴Percentage P retention = 100% - [100% × (Cr concentration in feed ÷ Cr concentration in feces) × (P concentration in feces ÷ P concentration in feed)].

⁵Percentage Ca retention = 100% - [100% × (Cr concentration in feed ÷ Cr concentration in feces) × (Ca concentration in feces ÷ Ca concentration in feed)].

⁶A: an NC diet containing 0.2% AP and 0.8% Ca; B: an LPC diet containing 0.28% AP and 0.8% Ca; C: an NC diet plus 0.35% KH₂PO₄ to provide 0.2% AP and 0.8% Ca; D: a corn-soybean meal-type diet containing LPC, 0.45% AP, and 1% Ca; E: a corn-soybean meal-type diet containing NC plus KH₂PO₄ to provide 0.45% AP and 1% Ca.

mented with inorganic P had similar Ca retention to those fed LPC. These results are in contrast with the findings of Veum et al. (1999), who reported that Ca retention in pigs fed an LPC diet was higher than in pigs fed an NC diet supplemented with inorganic P to match the AP level in the LPC diet. In addition, Ertl et al. (1998) reported that chicks fed the LPC had significantly higher bone and blood Ca compared with chicks fed NC supplemented with inorganic P.

Results of the current study also show that Ca retention was not affected by substituting LPC for NC in typical corn-soybean meal-type diets. Similar results were also reported by Veum et al. (1999). It is known that the Ca:AP ratio in poultry diets plays an important role in both Ca and P absorption. A wide Ca:AP ratio lowers Ca and P absorption, especially if the diet is marginal in both elements. The current Ca:AP ratio recommended by the NRC for starting broiler chicks is approximately 2.2:1 (NRC, 1994), which is the ratio used in Diets D and E. The wide Ca:AP ratio in Diet A (4:1), as well as the marginal Ca and AP present in the diet, may have adversely affected Ca absorption. The in-

creased Ca retention in Diets B and C could be a result of a narrower Ca:AP ratio, as a consequence of increased AP content in these diets. In the current study, lower Ca retention was observed in chicks fed typical corn-soybean meal-type diets compared with chicks fed other diets. The main difference between these diets is that the glucose, casein, and gelatin used in Diets A, B, and C was replaced by soybean meal in Diets D and E. These diets contained an additional source of phytate from soybean meal; it is known that phytate binds divalent cations, including Ca, and renders them unavailable for absorption. It is speculated that additional phytate from soybean meal may have adversely affected Ca retention.

Results of the in vitro study showed that in vitro P release from LPC was 2.6 times greater than the P release from NC, again suggesting that P in LPC is more available than P in NC. Phosphorus release values obtained from the in vitro study are consistent with the chemical analysis data. The in vitro study showed that P in NC and LPC was 23 and 65% available, respectively. The chemical analysis indicated that nonphytate P content in NC and LPC was 20 and 69%, respectively. These data suggest that the in vitro procedure used in the current study was effective in detecting in vivo P availability. Similar procedures have also been used to predict the P availability from a variety of feed ingredients for swine and turkeys, and appear to be effective in predicting P availability in plant ingredients (Zyla et al., 1995; Liu et al., 1998).

In summary, results of the present studies clearly demonstrate that P in LPC was more available than P in NC, and that reducing the phytate content in LPC did not compromise its nutritional value. Results also indicate that the amount of P excreted in poultry waste could be substantially reduced by substituting LPC for NC. It is estimated that excretion of P would be reduced by 55,000

TABLE 6. In vitro P release from wild-type and low-phytate corn¹

Item	TP ²	P release
	(%)	(mg/kg)
Corn type ³		
NC	0.24	543 ^b
LPC	0.22	1,420 ^a
Pooled SEM		55

^{a,b}Values are significantly different (*P* < 0.05).

¹Each value represents the mean of six replicates.

²TP = total P; values in TP column are analyzed values determined prior to in vitro digestion.

³NC = wild-type corn; LPC = low-phytate corn.

t/yr through the replacement of NC with LPC, assuming that poultry excretes 250,000 t P annually in the U.S. (Cromwell, 1994). In addition, the in vitro data agree with the increased P availability in LPC observed in the in vivo study, suggesting that the in vitro procedure used in the current study is valid for predicting P availability in corn.

ACKNOWLEDGMENTS

The authors would like to express their appreciation to Pioneer Hi-Bred International for supplying the corn used in this study. This study was supported in part by an USDA-CSREES Fund for Rural Project (Agreement No. 98-1A-0353-283).

REFERENCES

- AOAC, 1970. Official Methods of Analysis. 11th ed. Association of Official Analytical Chemists, Washington, DC.
- AOAC, 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Washington, DC.
- Cromwell, G. L., 1994. Diet formulation to reduce the nitrogen and phosphorus in pig manure. *In: Nutrient Management Symposium Proceedings*, Chesapeake Bay Commission, Harrisburg, PA.
- Cromwell, G. L., J. L. Pierce, H. L. Stillborn, D. W. Rice, D. S. Ertl, and V. Raboy, 1998. Bioavailability of phosphorus in low-phytic acid corn for chicks. *Poultry Sci.* 77(Suppl. 1):117.
- Ertl, D. S., K. A. Young, and V. Raboy, 1998. Plant genetic approaches to phosphorus management in agricultural production. *J. Environ. Qual.* 27:299–304.
- Liu, J., D. R. Ledoux, and T. L. Veum, 1998. In vitro prediction of phosphorus availability in feed ingredients for swine. *J. Agric. Food Chem.* 46:2678–2681.
- NRC, 1994. *Nutrient Requirements of Poultry*. 9th rev. ed. National Academy Press, Washington, DC.
- Raboy, V., D. B. Dickinson, and F. E. Below, 1984. Variation in seed total phosphorus, phytic acid, zinc, magnesium, and protein among lines of Glycine max and G. soja. *Crop Sci.* 24:431–434.
- Raboy, V., D. B. Dickinson, and M. G. Neuffer, 1990. A survey of maize kernel mutants for variation in phytic acid. *Maydica* 35:383–390.
- SAS Institute Inc., 1996. *SAS User's Guide: Statistics*. SAS Institute Inc., Cary, NC.
- Snedecor, G. W., and W. E. Cochran, 1967. *Statistical Methods*. 6th ed. The Iowa State University Press, Ames, IA.
- Sugiura, S. H., V. Raboy, K. A. Young, F. M. Dong, and R. W. Hardy, 1999. Availability of phosphorus and trace elements in low-phytate varieties of barley and corn for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 170:285–296.
- Veum, T., D. W. Bollinger, J. Smith, L. Harmon, D. R. Ledoux, V. Raboy, and D. S. Ertl, 1999. Low phytic acid corn improves calcium and phosphorus utilization for growing pigs. *Animal Sciences Departmental Report*, University of Missouri, Columbia, MO.
- Zyla, K., D. R. Ledoux, A. Garcia, and T. L. Veum, 1995. An in vitro procedure for studying enzymic dephosphorylation of phytate in maize-soybean feeds for turkey poults. *Br. J. Nutr.* 74:3–17.