

# Effect of Microbial Phytase and Citric Acid on Phosphorus Bioavailability, Apparent Metabolizable Energy, and Amino Acid Digestibility in Distillers Dried Grains with Solubles in Chicks

C. Martinez-Amezcuca, C. M. Parsons,<sup>1</sup> and D. H. Baker

*Department of Animal Sciences, University of Illinois, Urbana 61801*

**ABSTRACT** Three experiments were conducted to evaluate the effectiveness of OptiPhos phytase and citric acid for releasing the P that is not bioavailable in distillers dried grains with solubles (DDGS). The effect of OptiPhos phytate on AME<sub>n</sub> and amino acid digestibility was also determined. New Hampshire × Columbian chicks were fed experimental diets from 8 to 21 d of age. For Experiment 1, a basal P-deficient diet contained 40% DDGS plus supplemental amino acids, and DDGS was the only source of P. The basal diet was then supplemented with 1,000 or 10,000 phytase units (FTU)/kg of OptiPhos phytase or with 0.2% of P from KH<sub>2</sub>PO<sub>4</sub>. In Experiment 2, a slope-ratio chick growth and tibia ash assay used a P-deficient soybean meal basal diet, and it was found that the bioavailability of P in DDGS was 67%. For Experiment

3, a P-deficient basal diet with 30% DDGS plus supplemental amino acids was supplemented with 0.05 or 0.1% P from KH<sub>2</sub>PO<sub>4</sub>, with 3% citric acid, or with 1,000 or 10,000 FTU/kg of OptiPhos phytase. In Experiment 1, both 1,000 and 10,000 FTU/kg of phytase increased tibia ash but had no effect on AME<sub>n</sub>. Both 10,000 phytase units (FTU)/kg of phytase and supplemental P increased digestibility of amino acids. In Experiment 3, supplemental phytase and citric acid increased tibia ash (mg/tibia), and it was estimated that phytase and citric acid could release from 0.04 to 0.07% P from DDGS. In terms of bioavailability coefficients, the bioavailability of the P in DDGS was increased from 62 to 72%. These results indicate that phytase and citric acid increase the bioavailability of P in DDGS, but phytase at 1,000 FTU/kg had no consistent effect on AME<sub>n</sub> and amino acid digestibility.

**Key words:** phytase, citric acid, distillers dried grains with solubles, phosphorus, poultry

2006 Poultry Science 85:470–475

## INTRODUCTION

Distillers dried grains with solubles (DDGS) is a corn coproduct obtained from the dry-milling process of corn for ethanol production, using fermentation with the yeast *Saccharomyces cerevisiae* (Olentine, 1986; Davis, 2001). Traditionally, DDGS has been fed mainly to ruminants because of its high level of fiber and high variability in content and bioavailability of some nutrients, particularly lysine (Cromwell et al., 1993; Shurson, 2003). However, DDGS is a good source of P, containing 0.72% total P (NRC, 1994), and the bioavailability of P is higher than the 25 to 35% that is typical of most plant ingredients. For example, Martinez Amezcuca et al. (2004) recently reported that the bioavailability of P in 3 samples of DDGS averaged approximately 75% (i.e., relative to that in KH<sub>2</sub>PO<sub>4</sub>). Although the latter value is higher than that for most plant feedstuffs, approximately 25% of the P is not bioavailable for poultry. It would be beneficial to

identify feed additives that could improve the use of the nonavailable P in DDGS.

One potential feed additive is phytase enzyme. Phytase enzymes from microbial origin have been shown to improve P use in diets formulated with ingredients such as corn, wheat, and soybean meal. The efficacy of phytase for improving the bioavailability of P in DDGS has not been determined. Citric acid is another feed additive that has been shown to improve P use in chicks fed corn-soybean meal diets (Boling-Frankenbach et al., 2001b). Similar to phytase, the effectiveness of citric acid for increasing the bioavailability of P in DDGS has not been evaluated.

The primary object of this study was to determine if dietary phytase or citric acid can increase the bioavailability of P in DDGS. In addition, the effect of phytase on the AME<sub>n</sub> and amino acid digestibility of DDGS was also evaluated because some studies have indicated that this enzyme has positive effects on these criteria (Ravindran et al., 2001; Wu et al., 2003).

## MATERIALS AND METHODS

Two samples of DDGS were obtained from commercial ethanol plants. Dry matter and total P analysis was per-

©2006 Poultry Science Association, Inc.

Received July 5, 2005.

Accepted October 23, 2005.

<sup>1</sup>Corresponding author: poultry@uiuc.edu

formed by the AOAC (1995) procedures. The enzymatic activity of OptiPhos phytase was assayed prior to inclusion in the diets following the Han et al. (1999) procedure.

All animal housing, handling, and euthanasia procedures were approved by the University of Illinois Committee on Laboratory Animal Care. New Hampshire × Columbian chicks hatched at the poultry farm at the University of Illinois were used in the 2 experiments. Chicks were housed in thermostatically controlled starter battery cages with raised wire floors in an environmentally controlled room, and 24 h of light was provided daily. From d 1 to 8, chicks received a nutritionally complete corn and soybean meal starter diet (NRC, 1994) containing 23% CP and 3,100 kcal of ME/kg of diet. On d 8 after hatching following an overnight period of feed removal, chicks were weighed, wing-banded, and assigned to treatment groups so that their initial weights were similar among treatment groups. Three experiments were conducted, and in each experiment, 4 replicate groups of 5 chicks were assigned to each dietary treatment. The experimental diets were fed from 8 to 21 d of age, after which body weight gain, feed intake, and gain:feed were calculated. Feed and water were provided ad libitum. At the end of each experiment, chicks were killed with CO<sub>2</sub> gas and the right tibia bone was collected to obtain bone ash values. Tibias were pooled by replicate groups and autoclaved, and adhering tissue was removed. Bones were then dried for 24 h at 100°C, weighed, and then dry-ashed for 24 h in a 600°C muffle furnace. Ash weight was expressed as milligrams per tibia.

### **Experiment 1. Effect of Phytase on P Bioavailability, AME<sub>n</sub>, and Amino Acid Digestibility for DDGS**

The objective of this experiment was to evaluate if dietary phytase can release any or all of the unavailable P in DDGS and also if phytase would have a positive effect on AME<sub>n</sub> and amino acid digestibility. The P-deficient basal diet (Table 1) for this experiment was formulated to satisfy all of the nutrient requirements of the chicks except for P and to contain 40% DDGS as the only source of dietary P. A mixture of amino acids was included to meet digestible amino acid requirements based on Illinois ideal protein using the total amino acid content of the DDGS and the amino acid digestibility coefficients of the NRC (1994). The L-glutamic acid was added as a source of nonspecific amino N. Celite (2%) was added to the basal diet as a source of acid insoluble ash (AIA) for a digesta marker. The AIA in feed, ileal and excreta samples was measured using the procedures described by Vogtmann et al. (1975).

Four diets were evaluated. Diet 1 was the P-deficient basal diet. Diets 2, 3, and 4 were the basal plus 1,000 phytase units (FTU)/kg of OptiPhos phytase (United Feeds, Inc., Sheridan, IN), 10,000 FTU/kg of OptiPhos, and 0.9% KH<sub>2</sub>PO<sub>4</sub>, respectively, in place of dextrose and cornstarch. The OptiPhos phytase is an *Escherichia coli*-derived phytase that is classified as a 6-phytase and was

described in detail by Augspurger and Baker (2004). Diet 4 provided an additional 0.2% of bioavailable P as a positive-control treatment. In addition to growth performance and tibia ash, AME<sub>n</sub> and apparent ileal amino acid digestibility were determined for chicks in each treatment. The AME<sub>n</sub> was determined from excreta collection on d 21 to 22, and ileal amino acid digestibility was determined by the slaughter method at the end of the experiment. For the latter, chicks were killed with CO<sub>2</sub> gas, and the ileal region of the digestive tract was removed. The contents of the ileum were then collected by gentle squeezing and use of a water squirt bottle and then freeze-dried. Ileal contents and DDGS samples were analyzed for amino acids using ion exchange chromatography following hydrolysis in 6 N HCl for 24 h at 110°C (Spackman et al., 1958). Analyses of Met and cyst(e)ine were conducted following performic acid oxidation using the method of Moore (1963), except that samples were diluted with water and lyophilized to remove excess performic acid. Gross energy of feed and excreta was determined in an adiabatic bomb calorimeter standardized with benzoic acid. Nitrogen analyses for the AME<sub>n</sub> values were conducted using the macro-Kjeldahl procedure.

The statistical analysis was for completely randomized designs (SAS Institute, 1990). Apparent ileal amino acid digestibilities were calculated using AIA as the indigestible marker using the formula

Apparent digestibility (%) =

$$\frac{(\text{amino acid/AIA})_d - (\text{amino acid/AIA})_i}{(\text{amino acid/AIA})_d} \times 100,$$

in which (amino acid/AIA)<sub>d</sub> = ratio of amino acid or energy to acid-insoluble ash in the diet, and (amino acid/AIA)<sub>i</sub> = ratio of amino acid or energy to AIA in the ileal digesta.

The AME<sub>n</sub> values were calculated using the same formula except that the energy concentration in diet and excreta samples was used rather than amino acids, and the values were expressed as kilocalories per kilogram of DM.

### **Experiment 2. Determination of P Bioavailability in DDGS**

This experiment was conducted to determine bioavailability of P in the sample of DDGS intended for use in Experiment 3. The P-deficient basal soybean meal diet (Table 1) was the same as that used by Martinez Amezcua et al. (2004). The experiment consisted of 5 treatments. Diet 1 was the P-deficient basal diet that provided a calculated 0.1% nonphytate P. Diets 2 and 3 were the basal diet plus an additional 0.05 and 0.1% of P provided as KH<sub>2</sub>PO<sub>4</sub>, respectively. For diets 4 and 5, the basal diet was supplemented with 7 and 14%, respectively, of DDGS. The KH<sub>2</sub>PO<sub>4</sub> and DDGS additions were made in place of cornstarch and dextrose.

**Table 1.** Composition of P-deficient basal diets used in Experiments 1 to 3

Ingredient	Experiment 1	Experiment 2	Experiment 3
	(%)		
Cornstarch/dextrose (2:1 ratio)	to 100	to 100	to 100
DDGS <sup>1</sup>	40.00	—	30.00
Soybean meal	—	47.37	—
Soybean oil	5.00	5.00	4.00
Limestone	1.79	1.90	2.10
Salt	0.40	0.40	0.40
Vitamin mix <sup>2</sup>	0.20	0.20	0.20
Mineral mix <sup>3</sup>	0.15	0.15	0.15
K <sub>2</sub> CO <sub>3</sub>	0.42	—	0.81
Choline chloride (60%)	0.10	0.10	0.10
DL-Met	—	0.25	—
Amino acids <sup>4</sup>	10.65	—	14.47
NaHCO <sub>3</sub>	1.00	—	1.00
Celite	2.00	—	—
Silica flour	—	—	1.03
Bacitracin-MD premix <sup>5</sup>	0.042	0.042	0.042
Calculated analysis			
TME <sub>n</sub> , kcal/kg	3,267	3,260	3,200
CP, %	19.00	23.10	19.00
Ca, %	0.85	0.85	0.85
Total P (analyzed), %	0.30	0.32	0.20
Nonphytate P, % <sup>6</sup>	0.16	0.10	0.12

<sup>1</sup>DDGS = distillers dried grains with solubles.

<sup>2</sup>Provided (per kilogram of diet): thiamin HCl, 20 mg; niacin, 50 mg; riboflavin, 10 mg; D-Ca-pantothenate, 30 mg; vitamin B<sub>12</sub>, 0.04 mg; pyridoxine HCl, 6 mg; D-biotin, 0.6 mg; folic acid, 4 mg; menadione dimethylpyridinol bisulfite, 2 mg; cholecalciferol, 15 µg; retinyl acetate, 1,789 µg; ascorbic acid, 250 mg.

<sup>3</sup>Provided the following in milligrams per kilogram of diet: Mn, 75 from manganese oxide; Fe, 75 from iron sulfate; Zn, 75 from zinc oxide; Cu, 5 from copper sulfate; I, 0.75 from ethylene diamine dihydroiodide; Se, 0.1 from sodium selenite.

<sup>4</sup>The amino acid mixture for Experiment 1 provided (in %): DL-Met, 0.45; L-Glut, 6.3; L-Thr, 0.43; L-Trp, 0.11; L-Arg, 0.74; L-Lys-HCl, 1.13; L-Ile, 0.42; L-His-HCl, 0.21; L-Phe, 0.35; L-Leu, 0.07; L-Val, 0.44. The amino acid mixture for Experiment 3 provided (in %): DL-Met, 0.53; L-Glut, 8.6; L-Thr, 0.50; L-Trp, 0.12; L-Arg, 0.84; L-Lys-HCl, 1.19; L-Ile, 0.49; L-His-HCl, 0.28; L-Phe, 0.55; L-Leu, 0.34; L-Val, 0.53; Gly, 0.50.

<sup>5</sup>Contributed 13.75 mg of bacitracin methylene disalicylate/kg (3.28%).

<sup>6</sup>Estimated (NRC, 1994).

Data were initially analyzed using the ANOVA procedures of SAS software (SAS Institute, 1990) for completely randomized designs. Statistical significance of differences among individual treatments was assessed using the least significant difference test (Carmer and Walker, 1985). Data were further analyzed by multiple linear regression by regressing tibia bone ash (mg/chick) on supplemental P intake (mg/chick) from the KH<sub>2</sub>PO<sub>4</sub> or the DDGS samples. Bioavailability of P in DDGS relative to that of

KH<sub>2</sub>PO<sub>4</sub> as a standard was then estimated using the slope-ratio method (Finney, 1978).

### **Experiment 3. Effect of Phytase and Citric Acid on P Bioavailability in DDGS**

The results of Experiment 1 indicated that phytase increased the bioavailability of P in DDGS. Therefore, ex-

**Table 2.** Growth performance and tibia ash for chicks fed a P-deficient diet based on amino-acid-fortified distillers dried grains with solubles (DDGS), Experiment 1<sup>1</sup>

Dietary treatment	Weight gain (g)	Feed intake (g)	Gain: feed (g/kg)	Tibia ash (mg/chick)
1. Basal diet (40% DDGS; B)	288 <sup>b</sup>	481 <sup>a</sup>	599 <sup>b</sup>	453 <sup>c</sup>
2. B + 1,000 FTU <sup>2</sup> phytase/kg	297 <sup>b</sup>	482 <sup>a</sup>	612 <sup>ab</sup>	503 <sup>b</sup>
3. B + 10,000 FTU phytase/kg	299 <sup>b</sup>	486 <sup>a</sup>	621 <sup>a</sup>	519 <sup>b</sup>
4. B + 0.9% KH <sub>2</sub> PO <sub>4</sub> <sup>3</sup>	314 <sup>a</sup>	499 <sup>a</sup>	631 <sup>a</sup>	570 <sup>a</sup>
Pooled SEM	5	8	7	8

<sup>a-c</sup>Means within a column with no common superscript differ significantly ( $P < 0.05$ ) using the least significant difference test.

<sup>1</sup>Means represent 4 pens of 5 chicks per treatment; average initial weight was 93.7 g. Performance data are for the period 8 to 21 d posthatch.

<sup>2</sup>FTU = phytase units.

<sup>3</sup>Calculated to provide 0.2% supplemental P.

**Table 3.** Effect of phytase and supplemental P on ileal excreta AME<sub>n</sub> and ileal amino acid digestibility coefficients for chicks in Experiment 1

Measurement	Basal	Phytase (1,000 FTU <sup>1</sup> /kg)	Phytase (10,000 FTU/kg)	KH <sub>2</sub> PO <sub>4</sub> (0.9%)	SEM
Excreta AME <sub>n</sub> (kcal/kg of DM)	3,551	3,544	3,548	3,568	13
Thr digestibility (%)	63.8 <sup>c</sup>	67.5 <sup>b</sup>	75.2 <sup>a</sup>	75.8 <sup>a</sup>	1.05
Cyst(e)ine digestibility (%)	54.7 <sup>b</sup>	57.8 <sup>b</sup>	70.1 <sup>a</sup>	74.4 <sup>a</sup>	1.72
Val digestibility (%)	69.0 <sup>b</sup>	74.2 <sup>a</sup>	77.5 <sup>a</sup>	77.7 <sup>a</sup>	1.40
Met digestibility (%)	87.2 <sup>b</sup>	88.4 <sup>b</sup>	91.7 <sup>a</sup>	92.9 <sup>a</sup>	1.00
Ile digestibility (%)	71.5 <sup>b</sup>	73.7 <sup>b</sup>	79.8 <sup>a</sup>	81.0 <sup>a</sup>	1.25
Leu digestibility (%)	66.9 <sup>b</sup>	71.3 <sup>b</sup>	79.2 <sup>a</sup>	81.0 <sup>a</sup>	1.65
Tyr digestibility (%)	57.1 <sup>b</sup>	63.6 <sup>b</sup>	73.6 <sup>a</sup>	77.0 <sup>a</sup>	2.09
Phe digestibility (%)	76.6 <sup>b</sup>	77.9 <sup>b</sup>	83.3 <sup>a</sup>	84.8 <sup>a</sup>	1.24
His digestibility (%)	69.6 <sup>b</sup>	70.8 <sup>b</sup>	77.1 <sup>a</sup>	79.6 <sup>a</sup>	1.18
Lys digestibility (%)	74.9 <sup>b</sup>	77.8 <sup>b</sup>	83.2 <sup>a</sup>	83.8 <sup>a</sup>	1.45
Arg digestibility (%)	69.0 <sup>b</sup>	70.6 <sup>b</sup>	76.6 <sup>a</sup>	78.5 <sup>a</sup>	0.95
Trp digestibility (%)	69.8 <sup>b</sup>	72.5 <sup>b</sup>	81.2 <sup>a</sup>	81.7 <sup>a</sup>	2.27

<sup>a-c</sup>Means within a row with no common superscript differ significantly (*P* < 0.05) using the least significant difference test.

<sup>1</sup>FTU = phytase units.

periment 3 was conducted to determine if both phytase and citric acid would increase P bioavailability in DDGS and to quantify the amount of P released by the 2 feed additives.

Six diets or treatments were evaluated. Diet 1 was the basal P-deficient diet formulated to contain 30% of DDGS as the only source of P. As in Experiment 1, it was supplemented with a mixture of amino acids to meet all amino acid requirements as described for Experiment 1. Diets 2 and 3 were as diet 1 plus 0.05 or 0.10% P from KH<sub>2</sub>PO<sub>4</sub>, respectively. Diet 4 was as diet 1 plus 3% citric acid. Diets 5 and 6 were as diet 1 + 1,000 or + 10,000 FTU/kg OptiPhos enzyme, respectively. All additions were made in place of dextrose and cornstarch. Data from the experiment were analyzed as a completely randomized design. In addition, the tibia ash values for diets 1 to 3 were regressed on supplemental P intake (mg) to generate a regression equation. The amount of P released for diets 4 to 6 was then estimated using the standard-curve procedure, in which tibia ash values were substituted for Y in the regression equation to calculate the bioavailable P intakes and release values for treatments 4, 5, and 6.

## RESULTS AND DISCUSSION

The DDGS sample in Experiment 1 was analyzed to contain 0.76% total P, and the sample used in Experiments 2 and 3 was analyzed to contain 0.67% total P. The results of Experiment 1 are presented in Table 2. Addition of phytase generally had no significant effect on growth performance, but added P increased weight gain. Tibia ash, however, was significantly (*P* < 0.05) increased by both 1,000 and 10,000 FTU/kg phytase and by added P. These results indicated that phytase increased the bioavailability of P in the sample of DDGS evaluated in this experiment.

No effect of OptiPhos phytase on excreta AME<sub>n</sub> or ileal digestible ME<sub>n</sub> were observed (Table 3). Supplemental P also had no effect on ileal digestible energy or excreta AME<sub>n</sub>. These results agree with those presented by Augspurger and Baker (2004). However, these results do not agree with those presented by Ravindran et al. (2001), who reported improvements in AME<sub>n</sub> and ileal amino acid digestibility when phytase was added to both P-deficient and P-adequate diets. One possible reason for

**Table 4.** Growth performance and tibia ash for chicks fed a P-deficient soybean meal diet containing either supplemental P or distillers dried grains with solubles (DDGS), Experiment 2<sup>1</sup>

Dietary treatment	Weight gain (g)	Feed intake (g)	Gain:feed ratio (g/kg)	Tibia ash (mg/tibia)	Bioavailability of P <sup>2</sup> (%)
1. Basal (B)	240 <sup>d</sup>	386 <sup>c</sup>	620 <sup>c</sup>	257 <sup>d</sup>	
2. B + 0.05% P <sup>3</sup>	277 <sup>bc</sup>	438 <sup>b</sup>	632 <sup>bc</sup>	337 <sup>b</sup>	
3. B + 0.10% P <sup>3</sup>	303 <sup>a</sup>	464 <sup>a</sup>	652 <sup>a</sup>	420 <sup>a</sup>	100
4. B + 7% DDGS	274 <sup>d</sup>	423 <sup>b</sup>	648 <sup>ab</sup>	306 <sup>c</sup>	
5. B + 14% DDGS	286 <sup>b</sup>	439 <sup>b</sup>	653 <sup>a</sup>	345 <sup>b</sup>	62
Pooled SEM	4	6	5	8	

<sup>a-d</sup>Means within a column with no common superscript differ significantly (*P* < 0.05).

<sup>1</sup>Means represent 4 pens of 5 chicks per treatment; average initial weight was 96.5 g. Performance data are for the period 8 to 21 d posthatch.

<sup>2</sup>Calculated by the slope-ratio method. Multiple regression of tibia ash (Y; mg) on supplemental P intake (mg) from KH<sub>2</sub>PO<sub>4</sub> (X<sub>1</sub>) or DDGS (X<sub>2</sub>) yielded the equation: Y = 259.4 + 0.348 ± 0.0211X<sub>1</sub> + 0.215 ± 0.0237X<sub>2</sub>, R<sup>2</sup> = 0.94.

<sup>3</sup>From KH<sub>2</sub>PO<sub>4</sub>.

**Table 5.** Growth performance and tibia ash for chicks fed P-deficient diets based on amino-acid-fortified distillers dried grains with solubles (DDGS) and supplemented with P, citric acid, or phytase, Experiment 3<sup>1</sup>

Dietary treatment	Weight gain (g)	Feed intake (g)	Gain:feed (g/kg)	Tibia ash <sup>2</sup> (mg/chick)
1. Basal diet (B)	261 <sup>c</sup>	429 <sup>c</sup>	609	286 <sup>d</sup>
2. B + 0.05% P from KH <sub>2</sub> PO <sub>4</sub>	290 <sup>b</sup>	471 <sup>bc</sup>	616	385 <sup>b</sup>
3. B + 0.10% P from KH <sub>2</sub> PO <sub>4</sub>	320 <sup>a</sup>	545 <sup>a</sup>	590	520 <sup>a</sup>
4. B + 3% citric acid	265 <sup>bc</sup>	454 <sup>bc</sup>	585	314 <sup>c</sup>
5. B + 1,000 FTU <sup>3</sup> /kg of phytase	282 <sup>bc</sup>	484 <sup>b</sup>	584	313 <sup>c</sup>
6. B + 10,000 FTU/kg of phytase	280 <sup>bc</sup>	454 <sup>bc</sup>	617	327 <sup>d</sup>
Pooled SEM	10	15	24	9

<sup>a-d</sup>Means within a column with no common superscript differ significantly ( $P < 0.05$ ) using the least significant difference test.

<sup>1</sup>Means represent 4 pens of 5 chicks per treatment; average initial weight was 95.7 g. Performance data are for the period 8 to 21 d posthatch.

<sup>2</sup>Standard curve obtained from regression of tibia ash (Y; mg) on supplemental P intake (mg) from KH<sub>2</sub>PO<sub>4</sub> (X) yielded the equation:  $Y = 285.5 + 0.428X$ ,  $R^2 = 0.98$ .

<sup>3</sup>FTU = phytase units.

the difference between our study and that of Ravindran et al. (2001) is that the latter researchers used wheat-soybean-sorghum diets that contain much higher phytate levels than our DDGS diet. Thus, the effects of phytase on ME<sub>n</sub> might have been larger in their study because there was more substrate for the phytase enzyme.

For amino acid digestibility values, our data showed improvements in digestibility for some amino acids at 1,000 FTU/kg of OptiPhos, whereas at 10,000 FTU/kg there was a significant increase in digestibility for all amino acids (Table 3) compared with the basal diet. At first glance, these results support previous studies that reported improvements in nutrient digestibility by phytase supplementation (Ravindran et al., 2001; Shirley and Edwards, 2003; Johnston et al., 2004). However, the positive control diet with supplemental P in our study yielded increases in amino acid digestibility equal to or numerically higher than those obtained from 10,000 FTU/kg of OptiPhos phytase. Thus, our results suggest that phytase, per se, had no direct effect on amino acid digestibility, which agrees with the studies by Onyango et al. (2005),

Peter and Baker (2001), Boling-Frankenbach et al. (2001a), Snow et al. (2003), and Augspurger and Baker (2004). Thus, the effect of phytase on amino acid digestibility remains unclear and inconsistent among studies. It is possible that the positive effects of phytase and supplemental KH<sub>2</sub>PO<sub>4</sub> on amino acid digestibility were due to alleviating the P deficiency. Phosphorus is an important and necessary mineral for membrane function and active transporters such as the Na/K ATPase pump, which are essential for amino acid absorption.

Results of Experiment 2 are presented in Table 4. A linear ( $P < 0.01$ ) increase in weight gain and tibia ash (mg/chick) was observed as the P level was increased by adding KH<sub>2</sub>PO<sub>4</sub> or DDGS. The very high  $R^2$  value of 0.94 for the multiple regression indicated an excellent linear response to the KH<sub>2</sub>PO<sub>4</sub> and DDGS and also indicated that the nutrients other than P in the DDGS had no negative effect on growth and tibia ash. The estimated P bioavailability of this DDGS sample was 61.5%, which is slightly higher than the value of 54% calculated by NRC (1994) but below than the range of 69 to 102% observed

**Table 6.** Estimation of the increase in P bioavailability in distillers dried grains with solubles (DDGS) by OptiPhos phytase and citric acid, Experiment 3

Dietary treatment	Additional P released from DDGS <sup>1</sup>	Bioavailable P in DDGS	Bioavailability coefficient for P in DDGS <sup>2</sup>
	(%)		
1. Basal diet (30% DDGS; B)	—	0.412 <sup>3</sup>	62
2. B + 3% citrate	0.049	0.461	69
3. B + 1,000 FTU <sup>4</sup> /kg of phytase	0.043	0.455	68
4. B + 10,000 FTU/kg of phytase	0.072	0.484	72

<sup>1</sup>Values were calculated by substituting the tibia ash values for the treatments into the standard curve [standard curve obtained from regression of tibia ash (Y; mg) on supplemental P intake (mg) from KH<sub>2</sub>PO<sub>4</sub> (X) yielded the equation:  $Y = 285.5 + 0.428X$ ,  $R^2 = 0.98$ .] to estimate the increased bioavailable P intake. These values were then divided by the intakes of DDGS.

<sup>2</sup>Calculated by dividing the bioavailable P by the total P (0.67%) in DDGS.

<sup>3</sup>Calculated by multiplying the total P in DDGS (0.67%) by P availability of DDGS determined in Experiment 2 (61.5%) = 0.412%. The remaining values in the column were calculated by adding the 0.412% to the additional P release values.

<sup>4</sup>FTU = phytase units.

by Martinez Amezcua et al. (2004). The DDGS sample evaluated herein had a light golden yellow color that was similar in appearance to the sample that had a P bioavailability of 69% in the Martinez Amezcua et al. (2004) study. The P bioavailability value of 61.5% is within the range of 54 to 68% reported for 2 DDGS samples by Lumpkins et al. (2004).

As expected, growth performance and tibia ash were increased by supplementation of the basal diet with 0.05 and 0.10% P from  $\text{KH}_2\text{PO}_4$  in Experiment 3 (Table 5). Dietary addition of citric acid or phytase generally had no effect on growth performance but did increase tibia ash. By substituting the tibia ash values for the citric acid and phytase treatments into the regression equation for  $\text{KH}_2\text{PO}_4$  (Table 5), the amount of DDGS P released by citric acid and phytase were calculated (Table 6). Thus, it was estimated that citric acid and phytase released from 0.049 to 0.072% P from DDGS. Consequently, the bioavailable P content of DDGS was increased from 0.412 to 0.461, 0.455, and 0.484 by 3% citric acid, 1,000 FTU/kg of phytase, and 10,000 FTU/kg of phytase, respectively. These values indicate that citric acid and phytase released approximately 20 to 28% of the nonbioavailable P (0.26%) in DDGS. Of course, these values could vary among DDGS samples varying in P bioavailability. For example, as discussed earlier, previous work in our laboratory indicated that P bioavailability varied from 69 to 100% among 4 different DDGS samples. In conclusion, our results indicate that phytase can increase the bioavailability of P in DDGS but it seems to have no clear effect on  $\text{AME}_n$  or amino acid digestibility.

## REFERENCES

- AOAC. 1995. Official Methods of Analysis. 6th ed. Assoc. Off. Anal. Chem., Washington, DC.
- Augspurger, N. R., and D. H. Baker. 2004. High dietary phytase levels maximize phytate-phosphorus utilization but do not affect protein utilization in chicks fed phosphorus or amino acid-deficient diets. *J. Anim. Sci.* 82:1100–1107.
- Boling-Frankenbach, S. D., C. M. Peter, M. W. Douglas, J. L. Snow, C. M. Parsons, and D. H. Baker. 2001a. Efficacy of phytase for increasing protein efficiency ratio values of feed ingredients. *Poult. Sci.* 80:1578–1584.
- Boling-Frankenbach, S. D., J. L. Snow, C. M. Parsons, and D. H. Baker. 2001b. The effect of citric acid on the calcium and phosphorus requirements of chicks fed corn-soybean meal diets. *Poult. Sci.* 80:783–788.
- Carmer, S. G., and W. M. Walker. 1985. Pairwise multiple comparisons of treatment means in agronomic research. *J. Agron. Educ.* 14:19–26.
- Cromwell, G. L., K. L. Herkelman, and T. S. Stahly. 1993. Physical, chemical, and nutritional characteristics of distillers dried grains with solubles for chicks and pigs. *J. Anim. Sci.* 71:679–686.
- Davis, K. 2001. Corn milling, processing and generation of co-products. Paper presented at 62nd Minnesota Nutr. Conf. Minnesota Corn Growers Assoc. Tech. Symp., Bloomington, MN.
- Finney, D. J. 1978. Statistical Method in Biological Assay. 3rd ed. Charles Griffin and Company, Ltd., High Wycombe, Buckinghamshire, UK.
- Han, Y., D. B. Wilson, and X. G. Lei. 1999. Expression of an *Aspergillus niger* phytase gene (*phyA*) in *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* 65:1915–1918.
- Johnston, S. L., S. B. Williams, L. L. Southern, T. D. Binder, L. D. Bunting, J. O. Matthews, and B. M. Olcott. 2004. Effect of phytase addition and dietary calcium and phosphorus levels on plasma metabolites and ileal and total-tract nutrient digestibility in pigs. *J. Anim. Sci.* 82:705–714.
- Lumpkins, B. S., A. B. Batal, and N. M. Dale. 2004. Evaluation of distillers dried grains with solubles as a feed ingredient for broilers. *Poult. Sci.* 83:1891–1896.
- Martinez Amezcua, C., C. M. Parsons, and S. L. Noll. 2004. Content and relative bioavailability of phosphorus in distillers dried grains with solubles. *Poult. Sci.* 83:971–976.
- Moore, S. 1963. On the determination of cystine as cysteic acid. *J. Biol. Chem.* 238:235–237.
- NRC. 1994. Nutrient Requirements of Poultry. 9th ed. Natl. Acad. Press, Washington, DC.
- Olentine, C. G. 1986. Ingredient profile: Distillers feeds. Proceedings of Distillers Feed Conference. Organized by Distillers Feed Research Council. 41:12–24.
- Onyango, E. M., M. R. Bedford, and O. Adeola. 2005. Efficacy of an evolved *Escherichia coli* phytase in diets of broiler chicks. *Poult. Sci.* 84:248–255.
- Peter, C. M., and D. H. Baker. 2001. Microbial phytase does not improve protein-amino acid utilization in soybean meal fed to young chickens. *J. Nutr.* 131:1792–1797.
- Ravindran, V., P. H. Selle, G. Ravindran, P. C. H. Morel, A. K. Kies, and W. L. Bryden. 2001. Microbial phytase improves performance, apparent metabolizable energy, and ileal amino acid digestibility of broilers fed a lysine-deficient diet. *Poult. Sci.* 80:338–344.
- SAS Institute. 1990. SAS Users Guide: Statistics. Version 6, 4th ed. SAS Inst., Inc., Cary, NC.
- Shirley, R. B., and H. M. Edwards, Jr. 2003. Graded levels of phytase past industry standards improve broiler performance. *Poult. Sci.* 82:671–680.
- Shurson, J. 2003. DDGS suited for swine, may help ileitis resistance. *Feedstuffs*. May 26:11–13.
- Snow, J. L., M. W. Douglas, and C. M. Parsons. 2003. Phytase effects on amino acid digestibility in molted laying hens. *Poult. Sci.* 82:474–477.
- Spackman, D. H., W. H. Stein, and S. Moore. 1958. Automatic recording apparatus for use in the chromatography of amino acids. *Anal. Chem.* 30:1190–1206.
- Vogtmann, H., P. Frirter, and A. L. Prabuck. 1975. A new method of determining metabolizability of energy and digestibility of fatty acids in broiler diets. *Br. Poult. Sci.* 16:531–534.
- Wu, Y. B., V. Ravindran, and W. H. Hendriks. 2003. Effects of microbial phytase, produced by solid-state fermentation, on the performance and nutrient utilization of broilers fed maize-and wheat based diets. *Br. Poult. Sci.* 44:710–718.