Content and Relative Bioavailability of Phosphorus in Distillers Dried Grains with Solubles in Chicks

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ABSTRACT Total phosphorus analysis was performed on 20 samples of corn distillers dried grains with solubles (DDGS), and three experiments were conducted to determine the bioavailability of P in different samples of DDGS varying in Lys digestibility and heat processing (autoclaving). Relative bioavailability of P was estimated from tibia ash using the slope ratio method after chicks were fed a P-deficient corn-soybean meal diet supplemented with 0.05 or 0.10% P from KH_2PO_4 or supplemented with 2 levels of the test DDGS (7 to 25%). The mean total P value for the 20 DDGS samples was 0.73 ± 0.04% (SD), with an average dry matter value of 88 ± 0.8% (SD). In experiment 1, the bioavailability coefficient for P in a random sample of DDGS relative to KH_2PO_4 was 69%. In experiment 2, the relative bioavailabilities of P in low digestible Lys DDGS 1, low digestible Lys DDGS 2, and high digestible Lys DDGS 3 were 102, 82 and 75%, respectively ($P < 0.05$). For experiment 3, the P bioavailability coefficients for a light-colored nonautoclaved DDGS and the same DDGS autoclaved at 121°C and 124 pKa were 75 and 87%, respectively ($P < 0.05$). Our results showed that the total P content of DDGS was similar to the 0.72% value reported by the NRC (1994), but the relative P bioavailability is higher than the value estimated from NRC (1994) based on table values for total and nonphytate P content. Our results also indicated that there is substantial variability in P bioavailability among different DDGS samples and suggest that increased heat processing may increase the bioavailability of P in DDGS.

(*Key words*: distillers dried grains with solubles, phosphorus, bioavailability, chick)

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INTRODUCTION

Distiller dried grains with solubles (DDGS) is a corn coproduct obtained in the dry-milling process of corn to produce ethanol after fermentation with the yeast *Sacharomices cereviceae* (Olentine, 1986; Davis, 2001). Ethanol from grains is a relatively clean and renewable source of energy, and after the oil crisis in 1970, the interest in this fuel to reduce oil consumption has resulted in a large investment in facilities to produce ethanol. Moreover, the recent passage of the Renewable Fuels Standard will result in a large increase in ethanol and DDGS production in the next 10 yr. Traditionally, DDGS had been fed mainly to ruminants because of its high level of fiber and high variability in content and bioavailability of some nutrients, such as Lys (Cromwell et al., 1993; Shurson, 2003). Due to the large expected increase in DDGS production in the next decade, there will likely be a need to feed more DDGS to poultry and swine. Previous research has shown that DDGS can be successfully fed to poultry (Parsons et al., 1983; Olentine, 1986; Noll et al., 2001). In addition, new investments in ethanol technology and new facilities may provide a better quality DDGS (Whitney et al., 1999; Noll et al., 2001; Shurson, 2002). Thus, there is a need to reevaluate DDGS as an alternative ingredient for poultry diets.

One nutrient that particularly needs to be evaluated is P. The DDGS contains a substantial amount of total P (0.72%) (NRC, 1994). More recent data suggest that the total P in some sources of DDGS produced in new ethanol plants may be higher than 0.72% (Shurson, 2003). The bioavailability of P in DDGS is expected to be higher than in typical plant ingredients because of the fermentation process involved in ethanol production (Singsen et al., 1972; Mahgoub and El Hag, 1997; El Hag et al., 2002). However, there is very little information on the bioavailability of the total P in DDGS for poultry. Indeed, there are apparently no data published in scientific journals on the latter. The NRC (1994) reports that approximately 54% of the total P in DDGS is nonphytate P. Singsen et al. (1972) reported that P availability was 100% in DDGS for chicks. In studies with swine, Whitney et al. (2001) reported P availability in pigs ranged from 87.5 to 92.2%, which was similar to the value of 90% reported by Shurson (2003). The objective of the current

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Abbreviation Key: DDGS = distillers dried grains with solubles.

study was to determine the total P content and relative bioavailability of several DDGS samples for chicks.

MATERIAL AND METHODS

Total P Analysis

Twenty samples of DDGS were obtained from feed mills supplying feed directly for poultry operations. All of the samples were produced from different ethanol plants located in Minnesota. Dry matter analysis was performed using the AOAC (1995) procedure, and total P analysis was carried out by wet ashing (microwave digestion with HNO_3 and H_2O_2) and elemental analysis by inductively coupled plasma-atomic emission spectrometry.[2]

Chick Experiments for P Bioavailability

Three experiments were conducted with chicks to determine P bioavailability. All animal housing, handling, and euthanasia procedures were approved by the Institutional Animal Care and Use Committee. New Hampshire × Columbian male chicks were used in all experiments. The chicks were housed in thermostatically controlled starter battery cages with raised wire floors in an environmentally controlled room with light provided continuously. From d 1 to 8 posthatching, chicks received a nutritionally complete corn and soybean meal starter diet (NRC, 1994) containing 23% CP and 3,100 kcal of ME/kg. 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. Four replicate groups of 5 chicks each were fed experimental diets from 8 to 21 d of age. Feed and water were provided ad libitum. At the end of the experiments, all chicks were euthanized with CO_2 gas, and the right tibia bone was collected, autoclaved, cleaned, dried, weighed, and dry-ashed at 600°C to determine bone ash.

Experiment 1 consisted of 5 treatments. Diet 1 was a phosphorus-deficient basal diet that provided a calculated 0.1% nonphytate P (Table 1). Diets 2 and 3 were the same as the basal diet plus an additional 0.05 and 0.1% of P provided as KH_2PO_4, respectively. For diets 4 and 5, the same basal diet was supplemented with 12.5 and 25%, respectively, of a randomly selected DDGS (UMA 7 in Table 2). The KH_2PO_4 and DDGS additions were made in place of cornstarch and dextrose. Body weight gain, feed consumption, feed efficiency, and tibia bone ash in milligram per chicken and as a percentage were measured.

In experiment 2, 3 additional samples of DDGS that had previously been determined to vary in Lys digestibility were evaluated. Thus, the objective was to determine if P bioavailability was correlated with Lys digestibility. The digestibility coefficients of Lys had been determined earlier using the precision-fed cecectomized rooster assay (Douglas et al., 1997). The experimental design consisted of 9 dietary treatments. Diets 1 to 3 were the same as in experiment 1. For diets 4 to 9, each of the 3 samples of DDGS that varied in the coefficient for Lys digestibility was added to the basal diet at levels of 8 and 16% in place of cornstarch and dextrose. Diets 4 and 5 contained low digestible Lys DDGS 1 (64% Lys digestibility), diets 6 and 7 contained low digestible Lys DDGS 2 (61% Lys digestibility), and diets 8 and 9 contained high digestible Lys DDGS 3 (79% Lys digestibility). Growth performance and bone ash were measured as in experiment 1.

Experiment 3 was conducted to determine the effect of additional heat processing on the relative bioavailability of

TABLE 1. Composition of the P-deficient basal diet[1]

Ingredient	Amount (%)
Cornstarch/dextrose (2:1 ratio)	to 100
Soybean meal	47.37
Soybean oil	5.00
Limestone	1.20
Salt	0.40
Vitamin mix[2]	0.20
Mineral mix[3]	0.15
Choline chloride, 60%	0.10
DL-Met	0.25
Bacitracin-MD premix[4]	0.025

[1]Calculated to contain 23% CP; 3,200 kcal/kg TME_n ; 0.10% available P; and 0.58% Ca.

[2]Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 μg; DL-α-tocopheryl acetate, 11 IU; vitamin B_{12}, 0.01 mg; riboflavin, 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

[3]Provided as milligrams per kilogram of diet: manganese, 75 from $MnSO_4 \cdot H_2O$; iron, 75 from $FeSO_4 \cdot H_2O$; zinc, 75 from ZnO; copper, 5 from $CuSO_4 \cdot 5H_2O$; iodine, 0.75 from ethylene diamine dihydroiodide; selenium, 09.1 from Na_2SeO_3.

[4]Contributed 13.75 mg of bacitracin methylene disalicylate/kg (5.5%).

TABLE 2. Dry matter and total P content of 20 samples of distillers dried grains with solubles

Sample	Dry matter (%)	Total P (%)
UMA 1	87.1	0.76
UMA 2	86.9	0.77
UMA 3	88.0	0.75
UMA 4	87.7	0.74
UMA 5	87.8	0.77
UMA 6	88.2	0.74
UMA 7	87.5	0.72
UMA 8	87.3	0.75
UMB 1	87.7	0.76
UMB 2	87.4	0.77
UMB 3	89.0	0.74
UMB 4	87.5	0.74
UMC 1	87.8	0.73
UMC 2	88.4	0.74
UMC 3	88.9	0.73
UMC 4	87.9	0.73
UMD 1	86.9	0.67
UMD 2	88.3	0.67
UMD 3	85.3	0.62
UMD 4	87.6	0.69
Mean	87.7	0.73
SD	0.80	0.04

[2]ARL-3560, Simultaneous ICP-AES, Thermal Electron Corp., Waltham, MA.

TABLE 3. Growth performance from 8 to 21 d of age and tibia ash at 12 d for chicks, Experiment 1[1]

Dietary treatment	Weight gain (g)	Feed intake (g)	Gain:feed ratio (g/kg)	Tibia ash (mg/chick)[4]	Tibia ash (%)
1. Basal diet (B)	272[c]	377[d]	725	317[d]	31.5[d]
2. B + 0.05% P[2]	310[b]	425[c]	728	416[c]	35.8[c]
3. B + 0.10% P[2]	330[a]	449[ab]	735	482[b]	38.0[b]
4. B + 12.5% DDGS[3]	319[ab]	440[bc]	725	439[c]	36.5[c]
5. B + 25% DDGS[3]	331[a]	462[a]	718	524[a]	39.7[a]
Pooled SEM	5	7	10	12	0.4

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

[1]Means represent 4 pens of 5 chicks each per treatment; average initial weight was 103.8 g.

[2]From KH_2PO_4.

[3]DDGS = distillers dried grains with solubles. Sample was UMA 7 (Table 2).

[4]Multiple regression of tibia ash (Y; mg) on supplemental P intake (g) from KH_2PO_4 (X_1) or DDGS (X_2) yielded the equation: $Y = 329 + 353 \pm 36.2X_1 + 242 \pm 19.6X_2$, ($R^2 = 0.91$).

P in DDGS. A light, golden DDGS (suggestive of mild heat processing) was obtained from a commercial supplier, and then a portion of the sample was autoclaved at 121°C and 124 kPa for 75 min until the sample had become much darker (brown) in color. Seven dietary treatments were evaluated. Diets 1 to 3 were the same as in experiment 1. Diets 4 and 5 included 7 and 14% of the nonautoclaved DDGS, and diets 6 and 7 included 7 and 14% of the autoclaved DDGS, respectively. The DDGS was again added in place of cornstarch and dextrose. Growth performance and tibia ash were again measured.

Statistical Analysis

Data from all chick experiments were initially analyzed using the ANOVA procedure of SAS software (SAS Institute, 1990) for completely randomized designs. Statistical significance of differences among individual treatments were assessed using the least significant difference test (Carmer and Walker, 1985). In all chick experiments, data were further analyzed by multiple linear regression by regressing tibia bone ash (mg/chick) on supplemental P intake (g/chick) from the KH_2PO_4 or the DDGS samples. Bioavailability of P in DDGS relative to the KH_2PO_4 as a standard was then estimated using the slope-ratio method (Finney, 1978).

RESULTS AND DISCUSSION

Total P Content in DDGS Samples

The mean P content of the 20 DDGS samples was 0.73% on an air-dry basis (88% dry matter) and ranged from 0.62 to 0.77% (Table 2). The mean total P value was in generally good agreement with the total P value of 0.72% (93% dry matter basis) reported by NRC (1994). These total P values were slightly lower than those reported by Shurson (2003), who obtained a mean of 0.89% for total P on a dry matter basis. The variation in total P content among samples of DDGS could possibly be due to differences in level of P in the original corn source and factors that affect the efficiency and extent of starch fermentation during ethanol production, thereby influencing the residual starch and concentration of other nutrients in the final DDGS (Spiehs et al., 2002). Indeed, processing conditions such as temperature in the liquefaction process (that can range from 82 to 91°C), time of fermentation in the fermentation step (from 40 to 60 h), and variation in particle size among batches can affect efficiency of starch fermentation and residual starch in DDGS (Davis, 2001). In addition, Wu (1994) found almost 8% glycerol and 12% glucose (dry matter basis) in some samples of DDGS.

Relative Bioavailability of P in DDGS Samples

A linear increase in weight gain and tibia ash (mg/chick and %) was observed as the P level was increased by adding KH_2PO_4 or DDGS in all 3 experiments (Tables 3 to 5). In experiments 3 and 4, the tibia ash response for the highest level of DDGS in experiment 3 and the highest level of DDGS 1 in experiment 4 exceeded the KH_2PO_4 standard curve. However, statistical tests (Finney, 1978) showed no curvilinearity or lack of a common intersection effect, indicating that it was valid to include the highest level of the DDGS treatment in the multiple regression. These results were expected because previous results from our laboratory have shown that the response in tibia ash to P supplementation of the basal diet used herein is linear up to 0.2% supplemental P for chicks of the same age as in the current study (Augspurger et al., 2003; Augspurger and Baker, 2004).

In experiments 1 and 2, the estimated P bioavailability (relative to KH_2PO_4) varied greatly among different samples of DDGS, ranging from 69% for the UMA 7 sample in experiment 1 to 102% for the low digestible Lys DDGS 1 in experiment 2 (Tables 3 and 4). Regardless of the variation in P bioavailability among DDGS samples, our results indicate the relative bioavailability of P in DDGS is somewhat higher than that extrapolated from table values of the NRC (1994). That publication lists the total P of DDGS as 0.72% and the nonphytate P as 0.39%, suggesting that the bioavailability of P is 54%. The mean relative bioavailability of P for the 4 samples evaluated in experiment 1

TABLE 4. Growth performance from 8 to 21 d of age and tibia ash at 12 d for chicks, experiment 2[1]

Dietary treatment	Weight gain (g)	Feed intake (g)	Gain:feed ratio (g/kg)	Tibia ash (mg/chick)[4]	Tibia ash (%)
1. Basal	246[d]	382[d]	644	272[d]	34.7[f]
2. B + 0.05% P[2]	272[bc]	416[c]	652	349[c]	39.6[bcd]
3. B + 0.10% P[2]	280[bc]	424[abc]	672	397[b]	41.7[a]
4. B + 8% DDGS 1[3]	273[bc]	418[c]	649	348[c]	38.7[cde]
5. B + 16% DDGS 1[3]	293[a]	442[a]	670	434[a]	41.9[a]
6. B + 8% DDGS 2[3]	277[bc]	419[bc]	664	355[c]	38.3[de]
7. B + 16% DDGS 2[3]	282[abc]	432[abc]	645	389[b]	41.2[ab]
8. B + 8% DDGS 3[3]	270[c]	413[c]	658	332[c]	37.5[e]
9. B + 16% DDGS 3[3]	283[ab]	439[ab]	644	392[b]	40.3[abc]
Pooled SEM	4	7	5	10	0.6

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

[1]Means represent 4 pens of 5 chicks each per treatment; average initial weight was 93.3 g.

[2]From KH_2PO_4.

[3]DDGS = distillers dried grains with solubles. The 3 DDGS samples varied in Lys digestibility. The Lys digestibility coefficients for DDGS 1, 2, and 3 were 64.2, 61.2, and 78.8%, respectively, as determined by the precision-fed cecectomized rooster assay.

[4]Multiple regression of tibia ash (Y; mg) on supplemental P intake (g) from KH_2PO_4 (X_1) or DDGS 1, 2, and 3 (X_2–X_4 respectively) yielded the equation: $Y = 281 + 282 \pm 29.9X_1 + 289 \pm 24.5X_2 + 231 \pm 25.3X_3 + 212 \pm 24.6X_4$ ($R^2 = 0.84$).

and 2 was 82% (75% excluding the low digestible Lys DDGS 1 in experiment 2).

The reason for the variation in relative bioavailability of P among samples or sources of DDGS is unknown. Part or all of the variation may have been due to differences in the amount of total P that was present as phytin P among DDGS samples. This is not known because analyses for phytin P were not conducted. In addition, at least part of the variation could be due to processing conditions such as differences in time of fermentation, temperature, or both in the ethanol production process. Previous research with barley and wheat has shown that fermentation time and temperature during soaking have a direct effect on natural phytase activity (Carlson and Poulsen, 2003). Thus, it is possible that these factors could have an effect on the phytase activity provided by the *Sacharomices cereviceae* yeast utilized in the ethanol process. Another factor that could have an effect on P bioavailability is the degree, if any, of phytate destruction due to variation in the temperature and time of drying process in DDGS production. Mahgoub and Elhag (1997) reported that the phytic acid content of sorghum was reduced by 28% from mixing the sorghum with water (4:5 wt/vol) and cooking at 95°C, and Duhan et al. (2002) reported that phytic acid in pigeon peas was reduced by 12% from pressure cooking at 1.5 kg/cm^2. It is interesting that our DDGS sample containing the highest P bioavailability value was the low digestible Lys DDGS 1, a dark brown sample, suggestive of increased heat processing.

Experiment 3 was conducted to further evaluate the possibility that increased heat processing may increase the bioavailability of P in DDGS. The results of this trial are summarized in Tables 5 and 6. A linear increase in weight gain, feed intake, and tibia ash were again observed as P

TABLE 5. Growth performance from 8 to 21 d of age and tibia ash at 12 d for chicks, experiment 3[1]

Dietary treatment	Weight gain (g)	Feed intake (g)	Gain:feed ratio (g/kg)	Tibia ash (mg/chick)[4]	Tibia ash (%)
1. Basal diet (B)	216[c]	366[c]	592[b]	250[d]	27.0[d]
2. B + 0.05% P[2]	299[b]	445[b]	671[a]	329[c]	31.9[c]
3. B + 0.10% P[2]	342[a]	508[a]	672[a]	456[a]	37.5[a]
4. B + 7% DDGS 1[3]	297[b]	440[b]	674[a]	331[c]	31.3[c]
5. B + 14% DDGS 1[3]	332[a]	488[a]	680[a]	400[b]	34.0[b]
6. B + 7% DDGS 2[3]	302[b]	451[b]	669[a]	339[c]	31.5[c]
7. B + 14% DDGS 2[3]	347[a]	503[a]	691[a]	433[a]	36.3[a]
Pooled SEM	7	10	14	10	0.6

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

[1]Means represent 4 pens of 5 chicks each per treatment; average initial weight was 92 g.

[2]From KH_2PO_4.

[3]DDGS = distillers dried grains with solubles. The first DDGS was a light golden sample (total P = 0.76%), and the second sample was obtained from the same sample but was autoclaved at 121°C and 124 pKa for 75 min.

[4]Multiple regression of tibia ash (Y; mg) on supplemental P intake (g) from KH_2PO_4 (X_1) or DDGS 1 and 2 (X_2–X_3, respectively) yielded the equation: $Y = 251 + 395 \pm 21.8X_1 + 297 \pm 21.3X_2 + 342 \pm 20.4X_3$ ($R^2 = 0.95$).

TABLE 6. Relative bioavailability of P in distillers dried grains with solubles (DDGS), experiments 1, 2, and 3

Experiment	DDGS sample	Bioavailability coefficient[1] (%)	Total P content (%)	Bioavailable P content[2] (%)
1	UMA 7	69	0.72	0.49
2	Low digestible Lys 1	102[a]	0.74	0.75[a]
	Low digestible Lys 2	82[b]	0.72	0.59[b]
	High digestible Lys 3	75[b]	0.73	0.55[b]
3	Nonautoclaved DDGS	75[b]	0.76	0.57[b]
	Autoclaved DDGS	87[a]	0.76	0.67[a]

[a,b]Means within a column and experiment with no common superscript differ significantly ($P < 0.05$).

[1]Bioavailability of the P in DDGS relative to KH_2PO_4. Calculated by the slope ratio method using the multiple regression equations in the footnotes of Tables 3, 4, and 5.

[2]Calculated by multiplying the bioavailability coefficient by the total P content in the DDGS.

level increased by supplementing the diet with KH_2PO_4 or with nonautoclaved or autoclaved DDGS (Table 5). Bioavailability of P (relative to KH_2PO_4) in the autoclaved DDGS sample was approximately 15% higher ($P < 0.05$) than for the nonautoclaved DDGS sample. The bioavailability coefficients for the nonautoclaved and autoclaved DDGS samples were 75 and 87%, respectively, and the bioavailable P contents were 0.57 and 0.67%, respectively. These results suggest that increased heat processing may increase the bioavailability of P in DDGS even though it is well known that increased or excessive heat processing usually has a negative effect on protein solubility and amino acid content and digestibility (Anderson-Hafermann et al., 1993). Mahgoub and Elhag (1997) and Duhan et al. (2002) reported that the phytic acid in sorghum and pigeon peas could be partially destroyed or reduced by increased heating or cooking. In contrast, Ologhobo and Fetuga (1984) reported that autoclaving only slightly altered the phytate content of several legumes. The differences in results among studies could be due at least partially to the type of plant ingredient evaluated. The location of the phytates and phytase enzymes varies among plants and may affect their exposure and susceptibility to heating or cooking (Carlson and Poulsen, 2003). Further research on DDGS samples that have undergone different degrees of heat processing in commercial ethanol plants is needed to determine if P bioavailability is indeed affected by increased heating under commercial conditions.

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