# Nutrient Excretion, Phosphorus Characterization, and Phosphorus Solubility in Excreta from Broiler Chicks Fed Diets Containing Graded Levels of Wheat Distillers Grains with Solubles

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**ABSTRACT** Increased interest in ethanol production in North America has led to increased production of distillers dried grains with solubles (DDGS), the majority of which are fed to livestock. To determine the impact of including wheat DDGS in broiler diets on nutrient excretion and P characterization and solubility, 125 one-day-old male broiler chicks were fed wheat- and soybean meal-based diets containing 0, 5, 10, 15, or 20% wheat DDGS. There were 5 replicate pens per treatment, with 5 birds per pen arranged in a randomized block design. Apparent retention of both N and P were determined by using the indicator method. Nutrients excreted per kilogram of DM intake were also calculated. Characterization of excreta P was determined by $^{31}$P-solution nuclear magnetic resonance spectroscopy, and water-soluble P (WSP) was determined by extraction of excreta with deionized water. The apparent retention of both N ($P < 0.001$) and P ($P < 0.008$) decreased linearly with increasing inclusion rates of DDGS from 0 to 20%. The nutrient output per kilogram of DM intake increased linearly with increased DDGS inclusion rate for N ($P < 0.04$), P ($P < 0.0001$), and WSP ($P < 0.0003$). As the inclusion rate of DDGS increased, the P concentration in excreta increased ($P < 0.008$), whereas excreta phytate P concentrations decreased ($P < 0.01$), which led to an increase in WSP and the fraction of total P that was soluble. Because the inclusion of DDGS in poultry diets increased N and P output, as well as the solubility of P excreted, care should be taken when including high levels of DDGS in poultry diets, because increases in N and P excretion are a concern from an environmental standpoint.

**Key words:** distillers dried grain with solubles, broiler, phosphorus, nitrogen, phytate

2008 Poultry Science 87:2505–2511
doi:10.3382/ps.2008-00052

## INTRODUCTION

With the enactment of the nationwide Renewable Fuels Standard, the United States has made a commitment to increase the use of renewable fuels, such as ethanol and biodiesel. This legislation establishes a baseline for renewable fuel use, beginning with 4 billion gallons (15 billion liters) per year in 2006 and expanding to 7.5 billion gallons (28 billion liters) by 2012 (Renewable Fuels Association, 2006). The vast majority of the renewable fuel used will be ethanol, resulting in a doubling of the size of the domestic ethanol industry in the next 6 yr. Ethanol is now a ubiquitous component of the US transportation fuels market, because ethanol is now sold from coast to coast and is blended in 30% of the nation's gasoline. In 2005, ethanol production reached 4 billion gallons (15 billion liters), which is an increase of 17% from 2004 and 126% since 2001 (Renewable Fuels Association, 2006).

Distillers grains are a by-product of ethanol production and are typically sold for use as livestock feed. In 2005, ethanol dry mills produced 9 million metric tons of distillers grains, a 290% increase in production since 1999 (Renewable Fuels Association, 2006). The majority of distillers grains, most of which are dried and sold as distillers dried grains with solubles (**DDGS**), are fed to ruminants (75 to 80%), with the remainder fed to either swine (18 to 20%) or poultry (3 to 5%). Although the majority of grain used for ethanol production in the United States is corn, Canada plans to add 750 million liters to the country's annual ethanol production capacity and will rely in part on wheat to reach that goal. The result of this expansion of DDGS produced from both wheat and corn has been the need for more research into the feasibility of incorporating increasing


Received January 29, 2008.
Accepted August 2, 2008.
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amounts of these by-products into cattle, swine, and poultry rations.

Corn DDGS have been evaluated in both laying hen diets (Lumpkins et al., 2005) and broiler diets (Lumpkins et al., 2004), whereas Thacker and Widyaratne (2007) conducted feeding trials to determine the effects of wheat DDGS on the performance of broiler chicks. Although the inclusion of DDGS up to 15% did not seem to impair performance in either broilers or laying hens, there has been little published data addressing the potential environmental impacts of DDGS use in poultry diets. Increases in N and P excretion resulting from diet modification can increase the risk of nutrient enrichment of surface and ground waters when manures and litters are applied to land. The solubility of the P in manures and litter is also a concern, because Sharpley and Moyer (2000) found a strong correlation between water-soluble P (**WSP**) in manure and the amount of P leached from a soil after 5 simulated rainfall events. In addition, increases in N excretion can potentially contribute to increased emissions of ammonia from poultry houses and from land-applied manure or litter, which is a concern from an air quality perspective. It has been shown that inclusion of DDGS in cattle rations significantly increases N and P excretion because of corresponding increases in feed CP and P levels (Powers et al. 2006). Because DDGS contain greater levels of P than do corn or soybean meal, and can therefore reduce the amount of inorganic sources of P added to poultry diets, it has been suggested that inclusion of DDGS should not result in an increase in P excretion. However, this has not been tested. Therefore, the objectives of this study were to determine the effects of including wheat DDGS in broiler diets on N and P excretion as well as the characterization and solubility of P in excreta.

## MATERIALS AND METHODS

### Acquisition of Wheat DDGS

The wheat DDGS used in this study were obtained from the Husky/Mohawk ethanol plant located in Minnedosa, Manitoba, Canada. The wheat DDGS contained 35.7% CP, 4.6% ash, 5.4% ether extract, 33.2% neutral detergent fiber, 0.92% lysine, 1.13% threonine, and 1.50% methionine and cystine.

### Feeding Trial

The birds used in this experiment were cared for according to the guidelines of the Canadian Council on Animal Care (1993). The excreta analyzed in this experiment were obtained from a feeding trial conducted to determined the performance of broiler chicks fed graded levels of wheat DDGS (Thacker and Widyaratne, 2007). A total of 125 one-day-old male broiler chicks (Ross-308 line; Lilydale Hatchery, Wynyard, Saskatchewan, Canada) weighing an average of 52.8 ± 0.6 g were randomly assigned to 1 of 5 dietary treatments in a randomized block design. The experimental diets were based on wheat and soybean meal and contained 0, 5, 10, 15, or 20% wheat DDGS (Table 1). The experimental diets were formulated to supply 2,800 kcal/kg of ME, 1.1% lysine, 1.0% methionine and cystine, and 0.8% threonine. Lysine-HCl and DL-methionine were added to ensure that all diets provided a similar level of amino acids.

Canola oil was added to the diets containing wheat DDGS to compensate for its lesser energy content relative to soybean meal. The xylanase enzyme Avizyme 1310 (Danisco Animal Nutrition, Wiltshire, UK) was added to all diets to minimize the potential of anti-

**Table 1.** Composition of experimental diets fed to determine the effect of graded levels of wheat distillers dried grains with solubles (DDGS) on nutrient excretion from broiler chicks

| | Wheat DDGS (%) | | | | |
|---|---|---|---|---|---|
| Item (%, as fed) | 0 | 5 | 10 | 15 | 20 |
| Wheat | 66.02 | 63.25 | 60.45 | 57.67 | 54.88 |
| Soybean meal | 28.31 | 25.73 | 23.16 | 20.59 | 18.02 |
| Wheat DDGS | 0.00 | 5.00 | 10.00 | 15.00 | 20.00 |
| Canola oil | 1.01 | 1.33 | 1.65 | 1.97 | 2.29 |
| Dicalcium phosphate | 1.61 | 1.58 | 1.55 | 1.52 | 1.49 |
| Limestone | 1.41 | 1.43 | 1.46 | 1.48 | 1.50 |
| Salt | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Vitamin-mineral premix[1] | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Chromic oxide | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 |
| Lysine-HCl | 0.00 | 0.06 | 0.13 | 0.19 | 0.26 |
| DL-Methionine | 0.16 | 0.14 | 0.12 | 0.10 | 0.08 |
| Choline | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 |
| Avizyme[2] | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |

[1]Supplied per kilogram of diet: 11,000 IU of vitamin A (retinyl acetate + retinyl palmitate), 2,200 IU of vitamin $D_3$, 30 IU of vitamin E (DL-α-topheryl acetate), 2.0 mg of menadione, 1.5 mg of thiamine, 6.0 mg of riboflavin, 60 mg of niacin, 4 mg of pyridoxine, 0.02 mg of vitamin $B_{12}$, 10.0 mg of pantothenic acid, 6.0 mg of folic acid, 0.15 mg of biotin, 0.625 mg of ethoxyquin, 500 mg of calcium carbonate, 80 mg of iron, 80 mg of manganese, 10 mg of copper, 0.8 mg of iodine, and 0.3 mg of selenium.

[2]Avizyme (Danisco Animal Nutrition, Wiltshire, UK).

nutritional factors (i.e., the soluble xylans found in wheat) to affect broiler performance. All diets were supplemented with sufficient vitamins and minerals to meet or exceed the levels recommended by the NRC (1994). The experimental diets were provided in mash form (3-mm screen).

The chicks were housed in raised-floor battery cages (83.8 × 45.7 × 25.4 cm; Jamesway Manufacturing Co., Ft. Atkinson, WI) with mesh grate floors mounted over excreta collection trays. There were 5 birds per pen and 5 replicate pens per treatment. Feed and water were available ad libitum throughout the 21-d feeding trial. The battery brooder was maintained at a temperature of 35°C for the first week, with the temperature gradually reduced to 29°C by the end of the second week. Incandescent lighting was provided continuously during the experiment.

### Determination of Nutrient Retention

Chromic oxide (**Cr**, 0.35%) was added to all diets as an indigestible marker and was fed throughout the 21-d feeding trial. During the final 2 d of the experiment (morning and afternoon), clean excreta (free from feathers and feed) were collected from plastic liners placed in the collection trays underneath each pen of birds. The excreta from the 4 collections from each cage were pooled and then frozen for storage. Before analysis, the samples were dried in a forced-air oven at 55°C for 72 h, followed by fine grinding.

The apparent retention of P and N was determined by using the equations for the indicator method described by Schneider and Flatt (1975). Further, the total nutrients excreted per kg of DM intake (**DMI**) were calculated by using the ratio of Cr intake to Cr output (Dilger and Adeola, 2006):

$$\text{Nutrient output, g/kg of DMI} = \text{NcE} \times (\text{Cr}_{\text{diet}}/\text{Cr}_{\text{out}}),$$

where NcE is the concentration of the respective nutrient in the excreta; $Cr_{diet}$ is the initial Cr concentration in the diet, and $Cr_{out}$ is the concentration of Cr in the excreta.

### Chemical Analysis

Samples of the experimental diets and excreta were analyzed according to the methods of the Association of Official Analytical Chemists (1990). Analyses were conducted for moisture (method 930.15), CP (method 984.13), ash (method 942.05), and ether extract (method 920.39). Neutral detergent fiber was analyzed by using the method of Van Soest et al. (1991). The Ca and P contents of the experimental rations and excreta were determined by using the nitric-perchloric acid digestion method of Zasoski and Burau (1977), with Ca determined on a Perkin-Elmer Model 4000 Atomic Absorption Spectrophotometer (Perkin-Elmer, Waltham, MA; AOAC method 968.08) and total P determined colorimetrically (LKB Ultrospec III, Pharmacia, Cambridge, UK) by using a molybdovanadate reagent (AOAC, 1990; method 965.17). Chromic oxide was determined by the method of Fenton and Fenton (1979). Feed phytate P was determined by acid extraction, followed by HPLC analysis (HPLC 1100 series, Agilent Technologies, Wilmington, DE; Kwanyuen and Burton, 2005). Amino acid analysis was determined by HPLC (L-8800 Amino Acid Analyzer, Hitachi, Tokyo, Japan). All samples were hydrolyzed for 24 h at 110°C with 6 *N* HCl before analysis. Sulfur-containing amino acids were analyzed after cold formic acid oxidation for 16 h before acid hydrolysis.

Excreta samples were analyzed for WSP by shaking 1 g of dry excreta with 100 mL of deionized water for 1 h, filtering through a 0.45-μm membrane, and analyzing total WSP by inductively coupled plasma optical-emission spectrometry (4300 DV, Perkin-Elmer). Total N in excreta was determined by combustion of a 50-mg sample in a FlashEA1112 instrument (CE Elantech, Lakewood, NJ). The P composition of the excreta was determined by $^{31}$P- solution nuclear magnetic resonance spectroscopy as described by Leytem et al. (2007).

### Statistical Analysis

Statistical analysis was performed by using the Statistical Analysis System (SAS Institute, 1999). All variables were tested for normality by using the Shapiro-Wilk test with the PROC CAPABILITY procedure. Where results suggested nonnormality, variables were log-transformed before statistical analyses, with untransformed numbers presented in the text. All data were analyzed as a 1-way ANOVA by using the GLM procedure. Where appropriate, means separation was carried out by using Tukey's HSD with an α level of 0.05. Statements of statistical significance were based on $P < 0.05$ unless otherwise stated.

## RESULTS AND DISCUSSION

The analyzed nutrient content of the experimental diets is presented in Table 2. Analyzed dietary CP, Ca, and P were consistent across diets, with an average of 24.83, 0.90, and 0.73%, respectively. Dietary neutral detergent fiber increased with increasing inclusion of DDGS from 10.59 (0% inclusion) to 13.43% (20% inclusion). Dietary phytate P decreased slightly with increasing dietary inclusion of DDGS from 0.24 (0% inclusion) to 0.22% (20% inclusion).

The apparent retention of both N ($P < 0.001$) and P ($P < 0.008$) decreased linearly with increasing DDGS inclusion (0 to 20%; Table 3) in the diets. At the greatest DDGS inclusion rate (20%), there was a corresponding decrease in N digestibility of 19% and P digestibility of 23% compared with the negative control diet (0% inclusion). Thacker (2006) also found a linear decrease in

**Table 2.** Chemical composition of experimental diets fed to broiler chicks to determine the effects of graded levels of wheat distillers dried grains with solubles (DDGS) on nutrient excretion[1]

| | Wheat DDGS (%) | | | | |
|---|---|---|---|---|---|
| Chemical analysis (%, as fed) | 0 | 5 | 10 | 15 | 20 |
| Moisture | 10.91 | 11.04 | 10.84 | 10.28 | 10.06 |
| CP | 25.38 | 24.59 | 24.23 | 25.24 | 24.73 |
| Lysine | 1.23 | 1.17 | 1.14 | 1.21 | 1.13 |
| Methionine + cystine | 1.00 | 1.00 | 0.99 | 0.98 | 1.02 |
| Threonine | 0.84 | 0.82 | 0.80 | 0.83 | 0.79 |
| Neutral detergent fiber | 10.59 | 10.04 | 11.76 | 12.57 | 13.43 |
| Ether extract | 2.52 | 2.72 | 3.57 | 3.78 | 4.32 |
| Ash | 5.80 | 5.37 | 5.79 | 5.23 | 5.75 |
| Ca | 0.93 | 0.89 | 0.90 | 0.87 | 0.92 |
| P | 0.73 | 0.68 | 0.77 | 0.70 | 0.79 |
| Phytate P | 0.24 | 0.24 | 0.23 | 0.23 | 0.22 |
| Nonphytate P | 0.49 | 0.44 | 0.54 | 0.47 | 0.57 |

[1]All chemical composition data are the results of a chemical analysis conducted in duplicate.

nutrient digestibility with increasing DDGS inclusion rates in growing-finishing diets fed to swine.

The nutrient output per kilogram of DMI increased linearly with increased DDGS inclusion rate for N ($P < 0.04$), P ($P < 0.0001$), and WSP ($P < 0.0003$). Total N excreted per kilogram of DMI increased by 19% at the 20% DDGS inclusion rate compared with the negative control diet (0% DDGS). Widyaratne and Zijlstra (2007) also found that total N excretion was greater in both wheat-corn and wheat DDGS diets (40% inclusion rate) compared with negative control corn and wheat diets fed to grower-finishing swine. In the present study, total P and WSP excreted per kilogram of DMI increased by 24 and 65%, respectively, at the 20% DDGS inclusion rate compared with the negative control diet. Widyaratne and Ziljstra (2007) found no significant differences in total P excretion among the DDGS and control diets fed to growing-finishing swine.

The inclusion rate of DDGS in the diet also had an influence on the P composition of the excreta (Table 4). As the dietary inclusion rate of DDGS increased from 0 to 20%, there was a 23% increase in phosphate P in excreta ($P < 0.008$), from 6.54 to 8.02 g of P/kg, and a concomitant 20% decrease in the phytate P in excreta ($P < 0.01$), from 7.05 to 5.65 g of P/kg. There was no significant difference between the phosphate monoester concentrations (this includes inositol phosphates other than phytate) for the various diets. The pyrophosphate concentrations increased with increasing dietary DDGS inclusion rates, although they constituted only 2% or less of total P. Because sequential extraction of broiler litter has shown that P compounds extracted in water are predominantly inorganic P and that the majority of phytate P is extracted only with stronger extractions, such as HCl or NaOH (Turner and Leytem, 2004), litters or excreta that have a lesser proportion of phytate P will have greater WSP concentrations.

The relationship observed previously between the phytate content of excreta and WSP was evident in the present study. As the dietary DDGS inclusion rates increased, phytate P concentrations in excreta decreased; therefore, the WSP concentration of the excreta increased by 35% (Figure 1a; $r^2 = 0.71$). This was also demonstrated by the ratio of WSP to total P in the excreta, which increased by 33% with increasing dietary DDGS inclusion rates in this study (Figure 1b; $r^2 = 0.72$). When we examined the relationship between phytate P concentrations in excreta and WSP, we see there is a strong negative correlation between the 2 (Figure 2a, $r^2 = 0.64$), as well as for excreta phytate P and the ratio of WSP to total excreta P (Figure 2b, $r^2 = 0.60$). Examination of the available literature re-

**Table 3.** Apparent retention (%) of N and P as well as N, P, and water-soluble P output (g/kg of DM intake) from broiler chicks fed diets containing graded levels of wheat distillers dried grains with solubles (DDGS)

| | Wheat DDGS (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| Item | 0 | 5 | 10 | 15 | 20 | SEM | *P*-value |
| Apparent retention (%) | | | | | | | |
| N | 58.77[a] | 52.02[ab] | 53.26[ab] | 51.13[ab] | 47.89[b] | 1.84 | 0.001 |
| P | 41.78[a] | 35.72[b] | 37.69[ab] | 34.85[b] | 32.07[b] | 1.39 | 0.008 |
| Nutrient output (g/kg of DM intake) | | | | | | | |
| N | 19.69[b] | 21.43[ab] | 20.39[ab] | 22.25[ab] | 23.36[a] | 0.83 | 0.042 |
| P | 4.77[c] | 4.95[bc] | 5.42[b] | 5.08[bc] | 5.90[a] | 0.11 | <0.0001 |
| Water-soluble P | 1.72[c] | 2.18[bc] | 2.23[bc] | 2.32[ab] | 2.84[a] | 0.13 | 0.0003 |

[a–c]Means within a row lacking a common superscript differ significantly ($P \leq 0.05$).

vealed this same trend in broiler litter (Maguire et al., 2004; Toor et al., 2005) and manure from laying hens (Leytem et al., 2006).

Land application of poultry litter or manure can lead to N losses to the atmosphere via ammonia volatilization, which can be as great as 31% of total N applied to agricultural fields (Schilke-Gartley and Sims, 1993; Sharpe et al., 2004). Schilke-Gartley and Sims (1993) indicated that ammonia volatilization is related to the N content of manure; therefore, an increase in litter or manure N from high DDGS inclusion rates could increase ammonia lost to the atmosphere when manures are applied to land. In addition, litter or manure N can be lost from agricultural fields after application through runoff and leaching, thereby negatively affecting surface and ground waters (Edwards and Daniel, 1993; Pote et al., 2003).

Water-soluble P release to runoff from manure amended soils after rainfall has been found to vary considerably, primarily because of differences in the concentrations of WSP in the manure (Sharpley and Moyer, 2000; Kleinman et al., 2002; Vadas et al., 2004). In response to research that has demonstrated a strong relationship between manure and litter WSP and P losses in runoff, many states with areas of concentrated poultry production, such as Maryland, Arkansas, and North Carolina, have incorporated a measurement of WSP in manures or litters within their P loss assessment tools (Maryland Cooperative Extension, 2005; DeLaune et al., 2006; The North Carolina PLAT Committee, 2005). Because producers may be regulated based on manure WSP concentrations in these states, the increase in WSP associated with the use of DDGS in poultry diets may create a waste management issue for these producers.

Previous research has shown that DDGS are an acceptable ingredient for use in poultry diets, with recommended inclusion rates of 6% for broiler starter diets and up to 15% in growing-finishing and laying hen diets (Lumpkins et al. 2004; Lumpkins et al. 2005; Thacker and Widyaratne, 2007). Because supplemental P is becoming increasingly expensive, DDGS can replace dicalcium phosphate in feed formulations, thereby decreasing feed costs. However, the results of the current study indicate that as the inclusion rate of DDGS increases, retention of N and P decreases. This results in greater excretion of both N and P from birds fed diets containing DDGS, thereby causing concern from an environmental standpoint. Nitrogen excretion seems to be less affected by DDGS, with only high levels (20%) having significantly greater excretion over the control diet. However, all measures of P retention and excretion (digestibility, total P and WSP output) were more sensitive to dietary DDGS inclusion, and significant differences were seen, in some instances, at inclusion

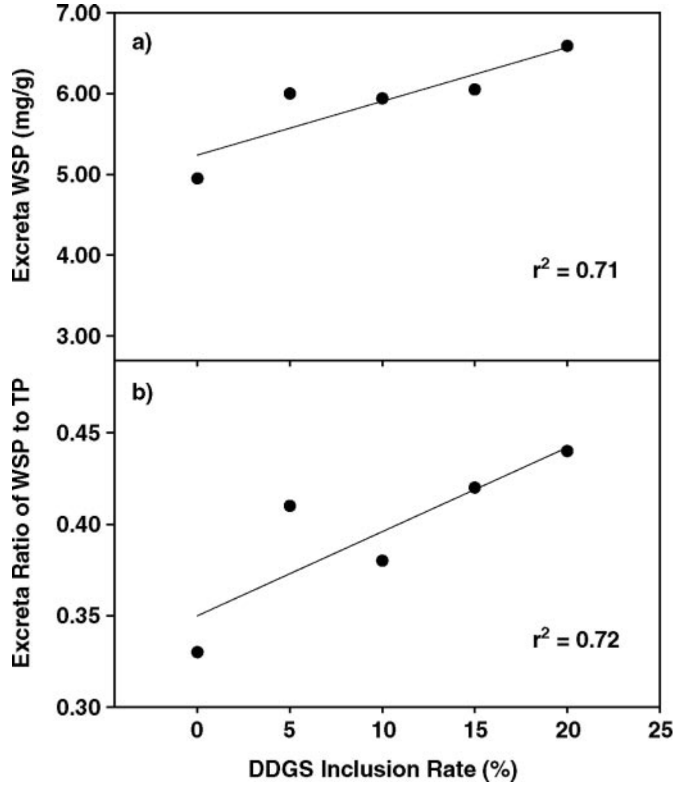


**Figure 1.** Relationship between inclusion of dietary distillers dried grains with solubles (DDGS) and a) excreta water-soluble P (WSP) and b) excreta ratio of WSP to total P (TP).

**Table 4.** Phosphorus characterization of poultry excreta from broiler chicks fed diets containing graded levels of wheat distillers dried grains with solubles (DDGS) as determined by NaOH-EDTA extraction and $^{31}$P-solution nuclear magnetic resonance spectroscopy

| Item | Total P | NaOH-EDTA-extractable P[1] | | | |
|---|---|---|---|---|---|
| | | Phosphate | Phosphate monoesters[2] | Phytate | Pyrophosphate |
| DDGS inclusion rate, g of P/kg of dry wt | | | | | |
| 0 | 14.38 | 6.54 (45)$^{b}$ | 0.56 (4)$^{a}$ | 7.05 (49)$^{a}$ | 0.22 (2)$^{b}$ |
| 5 | 13.88 | 6.91 (50)$^{ab}$ | 0.99 (7)$^{a}$ | 5.81 (42)$^{ab}$ | 0.16 (1)$^{ab}$ |
| 10 | 15.14 | 7.90 (52)$^{a}$ | 1.04 (7)$^{a}$ | 5.94 (39)$^{b}$ | 0.26 (2)$^{a}$ |
| 15 | 14.44 | 7.42 (51)$^{ab}$ | 0.94 (7)$^{a}$ | 5.81 (40)$^{b}$ | 0.26 (2)$^{ab}$ |
| 20 | 14.72 | 8.02 (54)$^{a}$ | 0.71 (5)$^{a}$ | 5.65 (39)$^{b}$ | 0.33 (2)$^{a}$ |
| SEM | 0.32 | 0.29 | 0.15 | 0.27 | 0.03 |
| $P > F$ | 0.12 | 0.008 | 0.18 | 0.01 | 0.004 |

[a,b]Means within a column lacking a common superscript differ significantly ($P \leq 0.05$).

[1]Values in parentheses are the percentages of the NaOH-EDTA-extracted P.

[2]Values for phosphate monoesters include all monoesters other than phytate.

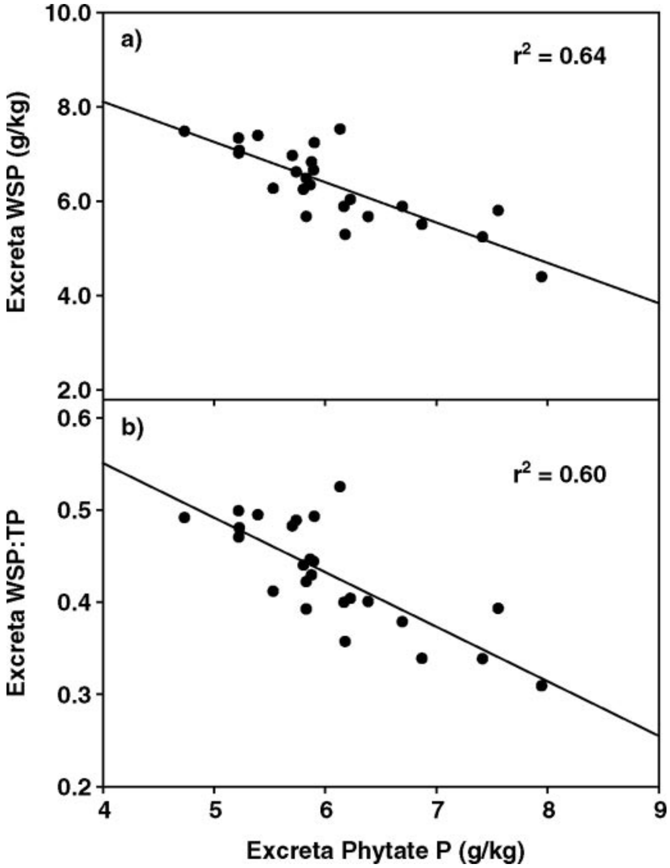


**Figure 2.** Relationship between excreta phytate P concentration and a) excreta water-soluble P (WSP) concentration and b) excreta WSP to total P (TP) ratio from broiler chicks fed graded levels of distillers dried grains with solubles.

rates as low as 5%. In addition, the WSP content of the excreta increased with increasing DDGS inclusion rates as well as the proportion of the total excreta P that was in a soluble form, which indicates that these excreta would have a greater risk of off-site P losses once applied to land.

## ACKNOWLEDGMENTS

The authors thank Alex Blumenfeld, University of Idaho (Moscow) for analytical support.

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