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Effects of production area and microbial phytase on the apparent and standardized total tract digestibility of phosphorus in soybean meal fed to growing pigs[1]

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ABSTRACT: An experiment was conducted to determine if the area in which soybeans are grown influences the concentration of P, phytate, and macro- and microminerals in the soybean meal (SBM) produced from the beans and, therefore, also influences the apparent total tract digestibility (ATTD) and the standardized total tract digestibility (STTD) of P in SBM. The second objective was to test the hypothesis that inclusion of microbial phytase will increase the ATTD and STTD of P in SBM regardless of where the beans were grown. Twenty sources of SBM were procured from crushing facilities located in different regions of the United States that were separated into 3 zones: 1) the northern growing area (Michigan, Minnesota, and South Dakota), 2) the eastern growing area (Georgia, Illinois, Indiana, and Ohio), and 3) the western growing area (Iowa, Missouri, and Nebraska). For each source of SBM, 2 diets based on cornstarch and SBM were formulated; one of these diets contained no microbial phytase and the other diet contained 500 units/kg of microbial phytase. Two hundred growing barrows (16.90 ± 1.79 kg initial BW) were individually placed in metabolism crates and allotted to a randomized complete block design with 40 diets and 5 replicate pigs per treatment. Feces were collected for 4 d after a 4-d adaptation period using the marker-to-marker procedure. Results indicated that there were no differences in concentration of Ca, P, phytate, and macro- and microminerals among SBM from the different zones. However, there was a tendency ($P = 0.055$) for an increase in concentration of nonphytate P in SBM from the western growing area (0.25%) compared with SBM from the northern growing area (0.23%) and the eastern growing area (0.23%). There were no differences in feed intake, absorbed P, ATTD of P, STTD of P, Ca intake, Ca output, or ATTD of Ca for pigs fed SBM from the 3 zones. However, there was a tendency (P = 0.066) for an increase in P intake and P output from pigs fed SBM from the western growing area compared with pigs fed SBM from the northern growing area. There was an increase ($P < 0.05$) in absorbed P, ATTD and STTD of P, and ATTD of Ca if microbial phytase was included in the diets, but the quantity of P and Ca that was excreted in the feces decreased ($P < 0.001$) if microbial phytase was used. Overall, no differences in ATTD and STTD of P exist among SBM produced in different areas of the United States, but microbial phytase will increase the digestibility of P in SBM.

Key words: apparent total tract digestibility, microbial phytase, phosphorus, pigs, soybean meal, standardized total tract digestibility

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INTRODUCTION

Between 60 and 70% of the P in cereal grains, oilseed meals, and many grain byproducts is unavailable to pigs because it is bound to phytate (Taylor, 1965; Jongbloed and Kemme, 1990; NRC, 2012). Soybeans contain 1 to 2% phytic acid; therefore, approximately two-thirds of P in soybeans is bound to phytate (Deak and Johnson, 2007; Paulsen, 2008; Rojas and Stein, 2012). To increase digestibility of P,

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microbial phytase may be included in the diets (Swick and Ivey, 1992). Increasing dietary concentrations of phytase resulted in a quadratic increase in apparent digestibility of P in soybean meal (SBM), with the greatest improvement observed if 500 units/kg of phytase were added to diets (Traylor et al., 2001).

The chemical composition of SBM is somewhat dependent on the area in which soybeans are grown (Grieshop et al., 2003), but it is not known if there are differences in the concentration of phytate among sources of SBM. The apparent total tract digestibility (**ATTD**) of P in distillers' dried grains with solubles is variable among sources (Pedersen et al., 2007), but no data for the digestibility of P among different sources of SBM have been published. Therefore, the objective of this experiment was to test the hypothesis that the area in which soybeans are grown may influence the concentration of P, phytate, and macro- and microminerals in the SBM produced from the beans and, therefore, also influence the ATTD and standardized total tract digestibility (**STTD**) of P in SBM. The second objective was to test the hypothesis that inclusion of microbial phytase will increase the ATTD and STTD of P in SBM.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment. Pigs used in the experiment were the offspring of G-Performer boars and Fertilis 25 females (Genetiporc, Alexandria, MN).

Soybean Meals, Animals, and Experimental Design

Twenty sources of SBM were procured from crushing facilities located in different regions of the United States (Tables 1 and 2). Approximately 500 kg of each source was collected from crushing plants in Georgia, Illinois, Indiana, Iowa, Michigan, Minnesota, Missouri, Nebraska, Ohio, and South Dakota and then subsampled, labeled, and stored. For analysis, the crushing plant locations were separated into 3 zones: 1) the northern growing area (Michigan, Minnesota, and South Dakota; 4 samples), 2) the eastern growing area (Georgia, Illinois, Indiana, and Ohio; 10 samples), and 3) the western growing area (Iowa, Missouri, and Nebraska; 6 samples). Two hundred growing barrows (16.90 ± 1.79 kg initial BW) were divided into 5 blocks with 40 pigs per block. Within each block, pigs were randomly allotted to 40 diets. Thus, there were 5 replicate pigs per diet. Pigs were individually placed in galvanized metal metabolism crates that were equipped with a feeder, a nipple waterer, a slatted floor, and a screen floor for total fecal collection.

Table 1. Locations of the crushing plants for the 20 sources of soybean meal used in the experiment

State	Zone[1]	Number of samples
Michigan	1	1
Minnesota	1	2
South Dakota	1	1
Georgia	2	1
Illinois	2	5
Indiana	2	2
Ohio	2	2
Iowa	3	2
Missouri	3	2
Nebraska	3	2
Total	3	20

[1]Zone 1 = the northern growing area (Michigan, Minnesota, and South Dakota); Zone 2 = the eastern growing area (Georgia, Illinois, Indiana, and Ohio); Zone 3 = western growing area (Iowa, Missouri, and Nebraska).

Diets, Feeding, and Sample Collection

Diets were based on a mixture of cornstarch, sucrose, soybean oil, and SBM, with SBM being the only source of P in the diets (Table 3). For each source of SBM, 2 diets were formulated; one of these diets contained no microbial phytase and the other diet contained 500 units/kg of microbial phytase (Quantum Blue G; AB Vista Feed Ingredients, Marlborough, UK). Vitamins and all minerals, except P, were included in all diets to meet or exceed the estimated nutrient requirements for growing pigs (NRC, 2012), and the same quantity of limestone was added to all diets. All diets were fed in a meal form. Feed consumption was recorded daily, and pigs were weighed at the beginning of the feeding period to determine feed allowance. Feed allotment was calculated as 3 times the estimated energy requirement for maintenance (i.e., 197 kcal of ME/kg$^{0.60}$; NRC, 2012). Feed was provided in 2 daily meals that were fed at 0700 and 1600 h, and pigs had ad libitum access to water throughout the experiment. Pigs were fed the experimental diets for 10 d. The initial 4 d were considered the adaptation period, and feces were collected twice daily during the following 4 d according to standard procedures using the marker to marker approach (Adeola, 2001). Fecal samples were stored at −20°C immediately after collection.

At the conclusion of the experiment, fecal samples were thawed and mixed within pig and diet and then dried in a 65°C forced-air drying oven and finely ground before analysis (Wiley Mill, model 4; Thomas Scientific, Swedesboro, NJ). Each source of SBM and all diets and fecal samples were analyzed in duplicate for DM (method 930.15; Horwitz and Latimer, 2007), and Ca and P were analyzed in these samples using inductively coupled plasma–optical emission spectroscopy (method 985.01 A, B, and D; Horwitz and Latimer,

Table 2. Analyzed nutrient composition of soybean meal (as-fed basis)

Item	Zone[1] 1	2	3	Average	SEM	*P*-value
Number of samples	4	10	6	–	–	–
DM, %	93.79	93.17	92.43	93.07	0.54	0.276
Ca, %	0.34	0.36	0.50	0.40	0.06	0.139
P, %	0.63	0.65	0.67	0.65	0.01	0.201
Phytate, %	1.43	1.49	1.50	1.48	0.04	0.474
Phytate-bound P,[2] %	0.40	0.42	0.42	0.42	0.01	0.551
Phytate-bound P, % of total P	63.75	65.30	63.43	64.43	0.99	0.289
Nonphytate P,[3] %	0.23	0.23	0.25	0.23	0.01	0.055
Non-phytate-bound P, % of total P	36.26	34.70	36.57	35.57	0.99	0.289
Mg, %	0.29	0.30	0.29	0.29	0.01	0.336
K, %	2.21	2.29	2.23	2.25	0.03	0.155
Na, %	0.02	ND[4]	0.01	0.01	0.01	0.234
Microminerals, mg/kg						
Co	0.10	0.15	0.15	0.14	0.03	0.507
Cu	25.64	33.31	34.32	32.08	4.38	0.407
Fe	213.13	162.68	209.49	186.81	31.52	0.384
Mn	41.62	35.55	38.25	37.57	3.28	0.435
Mo	4.12	4.05	2.67	3.65	1.20	0.619
Zn	45.50	47.60	52.45	48.43	4.93	0.639

[1]Zone 1 = the northern growing area (Michigan, Minnesota, and South Dakota); Zone 2 = the eastern growing area (Georgia, Illinois, Indiana, and Ohio); Zone 3 = western growing area (Iowa, Missouri, and Nebraska).

[2]Phytate-bound P was calculated as 28.2% of phytate (Tran and Sauvant, 2004).

[3]Nonphytate P = total P − phytate-bound P.

[4]ND = not detected.

2007) after wet ash sample preparation (method 975.03 B(b); Horwitz and Latimer, 2007). All sources of SBM were also analyzed in duplicate for Mg, K, Na, S, Cr, Co, Cu, Fe, Mn, Mo, and Zn (method 985.01 A, B, and C; Horwitz and Latimer, 2007) and for phytic acid (Ellis et al., 1977). Diets were also analyzed for phytase activity (method 200.12; Horwitz and Latimer, 2007).

Calculations and Statistical Analysis

Following chemical analysis, ATTD of P was calculated using the direct approach (NRC, 2012). The total tract basal endogenous loss of P was assumed to be 190 mg/kg DMI (NRC, 2012), and this value was used to calculate STTD of P using the following equation (NRC, 2012): STTD (%) = $[(P_{intake} - (P_{output} - \text{basal endogenous P loss})]/P_{intake} \times 100$.

Phytate-bound P was calculated as 28.2% of the analyzed phytate concentration (Tran and Sauvant, 2004). Non-phytate bound P was calculated by subtracting the phytate-bound P from the total P concentration in the SBM.

Normality of residuals and outliers were tested using the UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC). Data for the analyzed nutrient composition of SBM were analyzed using the MIXED procedure of SAS; the model included zone as a fixed effect. Data for the digestibility of P and Ca were analyzed as a 3 × 2 factorial using the MIXED procedure of SAS. The model included the fixed effects of zone, the level of phytase (0 or 500 units), and the zone × phytase interaction and the random effect of block. However, because the zone × phytase interaction was not significant for any variable, the interaction was removed from the final model, and main effects are presented. Least squares means for each zone were calculated using the LSMeans procedure in SAS, and if significant differences were detected, means were separated using the PDIFF option with the Tukey adjustment. Results were considered significant at $P \leq 0.05$ and considered a trend at $P \leq 0.10$.

RESULTS

No differences in concentrations of DM, Ca, P, phytate, phytate-bound P, percentage of total P bound to phytate, and nonphytate P as a percent of total P were detected among SBM from the different zones (Table 2). However, there was a tendency ($P = 0.055$) for an increase in concentration of nonphytate P in the western growing area (0.25%) compared with SBM from the northern growing area (0.23%) and the eastern growing area (0.23%). Likewise, there were no differences in concentrations of Mg, K, Na, S, and all

Table 3. Ingredient composition of experimental diets (as-fed basis)

	Diets[1]	
Ingredient, %	Without microbial phytase	With microbial phytase
Cornstarch	36.83	35.83
Soybean meal	43.66	43.67
Ground limestone	1.30	1.30
Soybean oil	2.98	2.97
Sodium chloride	0.39	0.39
Vitamin–mineral premix[2]	0.29	0.29
Phytase premix[3]	–	1.00
Sucrose	14.55	14.55

[1]Twenty diets without microbial phytase were formulated by using 20 different sources of soybean meal. Twenty additional diets were formulated using the same 20 sources of soybean meal and microbial phytase. Diets were formulated to contain 0.30% P.

[2]The vitamin–micromineral premix provided the following quantities of vitamins and microminerals per kilogram of complete diet: 11,136 IU vitamin A as retinyl acetate, 2,208 IU vitamin D_3 as cholecalciferol, 66 IU vitamin E as DL-alpha tocopheryl acetate, 1.42 mg vitamin K as menadione dimethylprimidinol bisulfite, 0.24 mg thiamin as thiamine mononitrate, 6.59 mg riboflavin, 0.24 mg pyridoxine as pyridoxine hydrochloride, 0.03 mg vitamin B_{12}, 23.5 mg d-pantothenic acid as d-calcium pantothenate, 44.1 mg niacin, 1.59 mg folic acid, 0.44 mg biotin, 20 mg Cu as copper sulfate and copper chloride, 126 mg Fe as ferrous sulfate, 1.26 mg I as ethylenediamine dihydriodide, 60.2 mg Mn as manganese sulfate, 0.3 mg Se as sodium selenite and selenium yeast, and 125.1 mg Zn as zinc sulfate.

[3]The phytase premix was prepared by mixing 24.75 kg of cornstarch and 250 grams of microbial phytase (Quantum Blue 5G [AB Vista Feed Ingredients, Marlborough, UK]; 5,000 units per gram). By including 1.00% of this mixture in the diet, the complete diet was expected to contain 500 units/kg of microbial phytase.

microminerals in SBM sources from the 3 growing areas. No microbial phytase was detected in the nonphytase diets (Table 4), but the concentrations of phytase in the diets containing microbial phytase were close to the expected value of 500 units/kg.

There were no differences in absorbed P, ATTD of P, STTD of P, Ca intake, Ca output, or ATTD of Ca among pigs fed SBM from the 3 zones (Table 5). However, there was a tendency ($P = 0.066$) for an increase in P intake and P output for pigs fed SBM from the western growing area compared with pigs fed SBM from the northern growing area. There was an increase ($P < 0.05$) in absorbed P, ATTD and STTD of P, and ATTD of Ca if microbial phytase was included in the diets, whereas the quantity of P and Ca excreted in the feces decreased ($P < 0.001$) if microbial phytase was used.

DISCUSSION

The sources of SBM used in this study were divided among 3 growing areas, and the SBM was procured from soybean crushing plants located within these areas. However, whereas the location of the crushing plants was known, the growing locations of the soybeans used in each crushing plant were not known, and therefore, it is possible that some of the soybeans that were crushed originated from a growing area that was different from the location of the crushing plant. However, it was expected that each crushing plant sourced the majority of the soybeans locally and, therefore, crushed beans that were grown in the same area as the location of the crushing plant.

The average concentration of P in SBM was less than values reported by the NRC (2012) but in agreement with values reported by de Blas et al. (2010), Rostagno et al. (2011), Rojas and Stein (2012), and Baker et al. (2014). Phytate-bound P and phytate-bound P as a percentage of total P were greater in this study compared with values reported by Rodríguez et al. (2013), but these values were not different from values reported by Rojas and Stein (2012). Phytate-bound P was also not different from values reported by de Blas et al. (2010), Rostagno et al. (2011), and the NRC (2012), but phytate-bound P as a percentage of total P was greater in this study compared with values reported by the NRC (2012). However, all sources of SBM contained high quantities of phytate, but the observation that there were no differences in P, phytate, or phytate-bound P among the 3 zones indicates that there is no regional variability in the concentrations of P and phytate. This observation is in agreement with data for canola meal indicating that there is very little effect of growing location on concentrations of P and phytate (Maison et al., 2015).

Concentrations of Ca in SBM from the northern and eastern growing areas were less than values reported by Rojas and Stein (2012) but in agreement with values reported by the NRC (2012). However, the concentration of Ca in SBM from the western growing area was not different from the value reported by Rojas and Stein (2012). The differences in concentrations of Ca may be the result of limestone being added to SBM at some crushing plants to improve flowability (Chiba, 2001).

Differences in mineral concentrations in the soil may impact the concentrations of minerals in soybeans and, therefore, also in SBM (Shacklette and Boerngen, 1984; Gustavsson et al., 2001). Differences in analytical procedures may also contribute to different values for minerals in feed ingredients. Nevertheless, the majority of mineral values in the SBM used in this experiment were in agreement with values reported by the NRC (2012). Values for Na observed in this experiment were less than values reported by the NRC (2012), but values for Cu and Fe were greater than values reported by the NRC (2012). Differences in Na may be observed in different sources of canola meal due to the addition of NaOH during refining (Newkirk, 2009), but no differences in Na concentrations among different sources of SBM were detected in this experiment, indicating that

Table 4. Analyzed DM, Ca, P, and phytase in experimental diets (as-fed basis)

	Zone[1]					
	1		2		3	
Item	0 FTU[2]/kg	500 FTU/kg	0 FTU/kg	500 FTU/kg	0 FTU/kg	500 FTU/kg
DM, %	92.99	93.33	92.77	92.87	93.05	92.78
Ca, %	0.77	0.72	0.74	0.71	0.72	0.72
P, %	0.28	0.28	0.30	0.28	0.30	0.29
Phytase, FTU/kg	<70	508	<70	515	<70	582

[1]Zone 1 = the northern growing area (Michigan, Minnesota, and South Dakota); Zone 2 = the eastern growing area (Georgia, Illinois, Indiana, and Ohio); Zone 3 = western growing area (Iowa, Missouri, and Nebraska).

[2]FTU = phytase units.

U.S. soybean crushing plants are likely not using NaOH during processing. Values for Mn were greater for SBM from the northern growing area compared with NRC (2012) values, but SBM from the eastern and western growing areas were close to reported values.

The ATTD and STTD of P observed in this experiment for SBM without microbial phytase were slightly greater than values reported by de Blas et al. (2010), Rostagno et al. (2011), and the NRC (2012) but were in agreement with data from Goebel and Stein (2011) and Rodríguez et al. (2013). The observation that there were no differences among the 3 zones in ATTD and STTD of P indicates that an average value for ATTD and STTD of P in SBM may be used regardless of the area in which the soybeans are grown.

Phytase is an enzyme that converts phytic acid to orthophosphate, inositol, and other phosphorus-inositol intermediates to increase the amount of P available to the pig (Swick and Ivey, 1992). The observed increase in ATTD of P when microbial phytase was added to the diet is in agreement with previous reports (Akinmusire and Adeola, 2009; Rodríguez et al., 2013). Addition of microbial phytase to SBM may increase ATTD and STTD of P by 16 to 27% (Almeida and Stein, 2010; Rojas and Stein, 2012; Rodríguez et al., 2013). In this experiment, the increase in ATTD and STTD of P was less than in previous experiments when microbial phytase was added; however, when microbial phytase was used, values for ATTD of P were not different from most previous values (Akinmusire and Adeola, 2009; Almeida and Stein, 2010; Rojas and Stein, 2012; Rodríguez et al., 2013).

The ATTD of Ca increases 8 to11% if microbial phytase is included in the diet (Selle et al., 2009; Goebel and Stein, 2011; Rojas and Stein, 2012; Rodríguez et al., 2013). This indicates that the Ca that is bound to phytic acid in SBM is released by phytase. However, the increase in ATTD of Ca in this experiment when microbial phytase was used was approximately 6% less than previous data, but values for ATTD of Ca obtained in this experiment are greater than those previous values. This may be due to a higher inclusion rate of limestone in the diets used in this experiment.

Conclusions

Results from this experiment indicate that concentrations of Ca, P, phytate, and macro- and microminerals in SBM are not influenced by the area within the United States where the SBM was produced. Concentrations of phytate were not different among growing areas, and no differences in ATTD or STTD of P were observed, indicating that growing area of soybeans did not influence the digestibility of P by

Table 5. Effects of zone and microbial phytase on apparent total tract digestibility (ATTD) of P and Ca and standardized total tract digestibility (STTD) of P in soybean meal produced in different areas of the United States

	Zone[1]					Phytase units/kg			
Item	1	2	3	SEM	*P*-value	0	500	SEM	*P*-value
Feed intake, g DM/d	782	770	790	14	0.193	769	793	13	0.021
P intake, g/d	2.36	2.39	2.47	0.05	0.066	2.41	2.40	0.04	0.812
P output, g/d	0.95	0.99	1.06	0.05	0.066	1.15	0.85	0.05	<0.001
Absorbed P, g/d	1.42	1.40	1.40	0.05	0.923	1.26	1.55	0.04	<0.001
ATTD of P, %	59.37	58.67	56.36	1.91	0.304	51.82	64.45	1.73	<0.001
STTD of P, %	65.67	64.81	62.50	1.91	0.273	57.91	70.74	1.73	<0.001
Ca intake, g/d	6.24	6.00	6.12	0.14	0.351	6.13	6.11	0.12	0.869
Ca output, g/d	1.44	1.52	1.63	0.12	0.161	1.71	1.35	0.11	<0.001
ATTD of Ca, %	76.40	74.62	73.26	1.61	0.171	71.75	77.77	1.49	<0.001

[1]Zone 1 = the northern growing area (Michigan, Minnesota, and South Dakota); Zone 2 = the eastern growing area (Georgia, Illinois, Indiana, and Ohio); Zone 3 = western growing area (Iowa, Missouri, and Nebraska).

pigs. The ATTD and STTD of P and the ATTD of Ca increased for SBM from all growing areas if microbial phytase was included in the diet.

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