Phosphorus digestibility and energy concentration of enzyme-treated and conventional soybean meal fed to weanling pigs

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Phosphorus digestibility and energy concentration of enzyme-treated and conventional soybean meal fed to weanling pigs[1]

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ABSTRACT: Two experiments were conducted to determine the apparent total tract digestibility (ATTD) of P and the concentration of DE and ME in enzyme-treated soybean meal (SBM) and in conventional soybean meal (SBM-CV). Phosphorus digestibility in 2 enzyme-treated SBM (HP-310 and HP-340) and in SBM-CV was measured using 36 barrows (initial BW: 21.9 ± 1.1 kg) that were housed in metabolism cages and randomly allotted to 6 diets with 6 replicates per diet. During production, HP-310 had been treated with an enzyme mixture containing no phytase, whereas HP-340 was treated with an enzyme mixture that contained exogenous phytase. Three diets containing HP-310, HP-340, or SBM-CV as the sole source of P were formulated. Three additional diets also contained HP-310, HP-340, and SBM-CV, but each of these diets was fortified with 500 units of microbial phytase. The ATTD of P in HP-310 and SBM-CV increased ($P < 0.05$) as phytase was included in the diet (from 59.8 to 77.7% for HP-310 and from 65.5 to 79.5% for SBM-CV), but the ATTD of P in HP-340 without and with phytase was not different ($P > 0.05$; 83.8 and 87.7%, respectively). There were no differences ($P > 0.05$) in the ATTD of P between HP-310 and SBM-CV, but the ATTD of P in HP-340 was greater ($P < 0.05$) than in the other 2 meals. The DE and ME in corn, 2 sources of enzyme-treated SBM (HP-200 and HP-310), and in SBM-CV were measured in the second experiment using 28 barrows housed in metabolism cages (initial BW: 16.8 ± 2.5 kg of BW). The process used to produce HP-200 is similar to that used to produce HP-310 except that HP-200 is exposed to the enzymes for a shorter period of time than HP-310. A corn-diet consisting of 96.45% corn and vitamins and minerals was formulated. Three additional diets were formulated by mixing corn and each source of SBM with vitamins and minerals. Pigs were randomly allotted to the 4 diets with 7 replicate pigs per diet, and urine and feces were collected quantitatively during the last 5 d of a 14-d feeding period. The concentration of DE in HP-200, HP-310, and SBM-CV was 4,333, 4,316, and 4,347 kcal/kg of DM, respectively. These values were not different ($P > 0.05$), but they were greater ($P < 0.05$) than the DE in corn (3,891 kcal/kg of DM). The concentration of ME was 3,780, 3,926, 3,914, and 3,980 kcal/kg of DM in corn, HP-200, HP-310, and SBM-CV, respectively. These values were not different ($P > 0.05$). It is concluded that enzyme treatment of SBM does not influence the digestibility of P or the concentration of DE and ME in the meals.

Key words: energy, enzyme-treated soybean meal, phosphorus, phytase, pig, soybean meal

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INTRODUCTION

Inclusion of soy proteins in diets fed to weanling pigs may negatively affect the pig by causing transient hypersensitivity, villi death, and malabsorption of nutrients (Li et al., 1990). Early-weaned pigs fed soybean meal (**SBM**)-based diets also have reduced growth performance compared with pigs fed diets containing no SBM because of the presence of antigens in SBM (Friesen et al., 1993). Soybean meal is, therefore, usually included in relatively small quantities in diets fed to newly weaned pigs, and most of the AA in these diets are provided by animal proteins despite the greater costs of animal proteins compared with SBM. However, it is possible that the high-priced animal proteins may be replaced by soy proteins if the antigens in soy protein are removed. Antigens in SBM may be removed by enzymatic treatment, and enzymatically treated SBM is now available to the US feed industry. Enzyme treatment results in removal of antigens in SBM as well as removal of sucrose and oligosaccharides, and the gross composition of enzyme-treated SBM is, therefore, different from that of conventional SBM (**SBM-CV**;

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Cervantes-Pahm and Stein, 2010). The digestibility of AA in enzyme-treated SBM has been measured (Min et al., 2004; Urbaityte et al., 2009; Cervantes-Pahm and Stein, 2010), but there is no information about the digestibility of P and energy in enzyme-treated SBM. The objective of this research, therefore, was to measure P digestibility and the concentration of DE and ME in enzyme-treated SBM and in SBM-CV and to test the hypothesis that enzyme treatment of SBM does not compromise P and energy digestibility despite the change in gross composition that is a result of the enzyme treatment process.

MATERIALS AND METHODS

The experimental protocols were reviewed and approved by the Animal Care and Use Committee at the University of Illinois.

Two experiments were conducted and SBM used in both experiments was obtained from a commercial company (Hamlet Protein A/S, Horsens, Denmark). Pigs used in the experiments were the offspring of Landrace boars that were mated to Yorkshire × Duroc females (Pig Improvement Company, Hendersonville, TN).

P Digestibility (Exp. 1)

Experiment 1 was designed to measure the apparent total tract digestibility (**ATTD**) of P in 2 sources of enzyme-treated SBM (HP-310 and HP-340) and in SBM-CV (Table 1). The 2 enzyme-treated SBM and SBM-CV were produced from the same batch of soybeans. The enzymatic treatment takes place in a continuous-flow system where SBM is exposed to the enzyme preparation for several hours. The difference between HP-310 and HP-340 is that HP-340 was treated with an enzyme mixture that included phytase (Rhonozyme, DSM Nutritional Products AG, Basel, Switzerland), whereas no phytase was included in the enzymes used to produce HP-310.

Three diets were formulated by mixing cornstarch, sugar, and each of the 3 sources of SBM (Tables 2 and 3). Three additional diets were formulated by adding 500 units of microbial phytase (Optiphos 2000, Enzyvia LLC, Sheridan, IN) to diets that were otherwise similar to the 3 aforementioned diets. Vitamins and all minerals, except P, were added to the diets to meet or exceed current requirement estimates for weanling pigs (NRC, 1998). No inorganic P was added to the diets, and the only source of P in the diets, therefore, was the P contributed by the SBM.

Thirty-six weanling barrows (initial BW: 21.9 ± 1.1 kg) were randomly allotted to the 6 diets with 6 replicate pigs per diet. Pigs were housed in metabolism cages that allowed for total collection of feces. The daily feed allowance was calculated as 3 times the maintenance energy requirement (i.e., 106 kcal of ME per $kg^{0.75}$; NRC, 1998) and divided into 2 equal meals. Water was available at all times. Pigs were fed their experimental diets for 14 d. The initial 7 d was an adaptation period to the diet. A marker (chromic oxide) was added to the morning meals on d 8 and 13. Fecal collection from each pig was initiated with the first appearance of the marker in the feces after d 8, whereas fecal collection ceased when the marker first appeared in the feces after d 13 (Petersen and Stein, 2006).

Fecal samples were stored at −20°C immediately after collection. At the conclusion of the experiment, fecal samples were dried in a forced-air oven and finely ground. All samples of SBM, diets, and feces were analyzed for DM by oven-drying duplicate samples at 135°C for 2 h (method 930.15; AOAC Int., 2007). Phosphorus and Ca were analyzed in these samples by the inductively coupled plasma spectroscopy procedure (method 985.01; AOAC Int., 2007) after wet-ash sample preparation (method 975.03; AOAC Int., 2007). Ingredients and diets were also analyzed for CP (method 990.03; AOAC Int., 2007) and AA (Hitachi Amino Acid Analyzer, model No. L8800, Hitachi High Technologies America Inc., Pleasanton, CA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Before analysis, samples were hydrolyzed with 6 *N* HCl for 24 h at 110°C [method 982.30 E(a); AOAC Int., 2007]. Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis [method 982.30 E(b); AOAC Int., 2007]. Tryptophan was determined after NaOH hydrolysis for 22 h at 110°C [method 982.30 E(c); AOAC Int., 2007]. All diets were also analyzed for phytase activity (Phytex Method, version 1, Eurofins, Des Moines, IA), and ingredients were analyzed for GE using bomb calorimetry (model 6300, Parr Instruments, Moline, IL). Benzoic acid was used as the internal standard for calibration. Additional analyses on ingredients included analysis for ether extract (method 2003.06; AOAC Int., 2007), ash (method 942.05; AOAC Int., 2007), ADF (method 973.18; AOAC Int., 2007), NDF (Holst, 1973), sucrose, stachyose, and raffinose (method 982.14; AOAC Int., 2007), and trypsin inhibitor activity (method Ba 12-75; AOCS Int., 2006). The concentration of β-conglycinin and glycinin was also measured in the 3 sources of SBM (Van Biert and Hessing, 1993).

After chemical analysis, the ATTD of P was calculated for each diet (Petersen and Stein, 2006). Because SBM was the only P contributing ingredient in the diets, the calculated ATTD for each diet also represents the ATTD of P in each source of SBM.

Data were analyzed using the Proc GLM procedure (SAS Inst. Inc., Cary, NC). An ANOVA was performed with source of SBM, level of phytase, and the interaction between SBM, and phytase as effects. Treatment means were separated using the LSD test in Proc GLM. The pig was the experimental unit for all analyses, and an α-value of 0.05 was used to assess significance among means.

Table 1. Chemical composition of enzymatically treated soybean meal and conventional soybean meal, as-fed basis

	Ingredient[1]			
Item	HP-200	HP-310	HP-340	SBM-CV
DM, %	89.70	91.28	90.98	87.32
GE, kcal/kg	4,435	4,473	4,446	4,159
CP, %	52.62	57.71	56.50	48.31
Ca, %	0.25	0.31	0.27	0.23
P, %	0.70	0.78	0.74	0.65
Ether extract, %	1.39	1.27	0.98	0.95
ADF, %	5.54	5.31	5.27	4.55
NDF, %	11.79	12.60	9.90	7.63
Ash, %	5.93	6.33	6.26	5.74
Trypsin inhibitor, TIU[2]/mg	3.10	2.40	1.80	5.70
β-Conglycinin, mg/kg	3	4	5	130,000
Glycinin, mg/kg	17,000	3,300	90	420,000
Sucrose, %	0.20	0.20	0.20	5.78
Stachyose, %	0.26	0.27	0.20	3.78
Raffinose, %	0.42	0.43	0.21	1.05
Indispensable AA, %				
Arg	3.88	4.05	3.96	3.63
His	1.41	1.44	1.43	1.27
Ile	2.62	2.70	2.65	2.36
Leu	4.25	4.42	4.32	3.88
Lys	3.24	3.54	3.59	3.17
Met	0.72	0.73	0.73	0.70
Phe	2.76	2.89	2.80	2.51
Thr	2.03	2.11	2.09	1.89
Trp	0.76	0.81	0.79	0.72
Val	2.69	2.74	2.74	2.42
Dispensable AA, %				
Ala	2.39	2.43	2.43	2.14
Asp	6.07	6.34	6.14	5.65
Cys	0.68	0.69	0.69	0.69
Glu	9.35	9.84	9.53	8.85
Gly	2.28	2.36	2.32	2.06
Pro	2.55	2.71	2.69	2.39
Ser	2.27	2.46	2.39	2.16
Tyr	1.90	2.01	1.95	1.78

[1]HP-200 (Hamlet Protein A/S, Horsens, Denmark) = short-time enzyme-treated soybean meal; HP-310 = enzyme-treated soybean meal; HP-340 = enzyme-treated soybean meal with phytase; and SBM-CV = conventional soybean meal that has not been enzyme treated.

[2]TIU = trypsin inhibitor units.

Energy Concentration (Exp. 2)

Experiment 2 was designed to measure the ATTD of energy and the concentration of DE and ME in SBM-CV and in 2 sources of enzyme-treated SBM (HP-200 and HP-310). The difference between HP-200 and HP-310 is that HP-200 is processed at a faster rate than HP-310 and soybean hulls are added to this meal before enzyme treatment. The concentrations of CP and AA are, therefore, reduced in HP-200 compared with HP-310. A corn diet that contained 96.45% corn and vitamins and minerals was formulated, and 3 additional diets were formulated by mixing corn, each source of SBM, and vitamins and minerals (Tables 4 and 5).

A total of 28 weanling barrows (initial BW: 16.8 ± 2.5 kg) were randomly allotted to the 4 diets with 7 replicate pigs per diet. Pigs were placed in metabolism cages that were equipped with a feeder and a nipple drinker, slatted floors, a screen floor, and urine trays, which allowed for the total, but separate, collection of urine and feces from each pig. The quantity of feed provided per pig daily was calculated as 3 times the estimated requirement for maintenance energy for the smallest pig in each replicate and divided into 2 equal meals. Water was available at all times. The experiment lasted 14 d. The initial 7 d was considered an adaptation period to the diet, and feces were collected during the next 5 d according to standard procedures using the marker-to-marker approach as explained for the P-digestibility experiment. Fecal samples were collected twice daily and immediately stored at −20°C. Urine was collected from d 8 to 13 in urine buckets over a preservative of 50 mL of 6 *N* HCl. Buckets were emptied twice daily, and 20% of the collected urine was stored at −20°C immediately after collection. At the conclusion of the experiment, urine samples were thawed and mixed within animal and diet, and a subsample was collected for analysis.

Table 2. Ingredient composition of experimental diets (as-fed basis), Exp. 1[1]

	Diet[2]		
Ingredient	HP-310	HP-340	SBM-CV
HP-310	38.00	—	—
HP-340	—	38.00	—
SBM-CV	—	—	44.00
Soybean oil	3.00	3.00	3.00
Ground limestone	0.70	0.70	0.70
Sucrose	15.00	15.00	15.00
Cornstarch	42.60	42.60	36.60
NaCl	0.40	0.40	0.40
Vitamin-mineral premix[3]	0.30	0.30	0.30

[1]Three additional diets were formulated by adding 0.025% Optiphos 2000 (Enzyvia, Sheridan, IN) to each diet at the expense of cornstarch. At this inclusion rate, Optiphos 2000 provides 500 units of phytase per kilogram of complete diet.

[2]HP-310 (Hamlet Protein A/S, Horsens, Denmark) = enzyme-treated soybean meal; HP-340 = enzyme-treated soybean meal with phytase; and SBM-CV = conventional soybean meal that has not been enzyme treated.

[3]The vitamin-micromineral premix provided the following quantities of vitamins and microminerals per kilogram of complete diet: vitamin A, 11,128 IU; vitamin D_3, 2,204 IU; vitamin E, 66 IU; vitamin K, 1.42 mg; thiamin, 0.24 mg; riboflavin, 6.58 mg; pyridoxine, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid, 23.5 mg; niacin, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

Fecal samples were dried in a forced-air oven and finely ground. The HP-200 was analyzed as described for the ingredients used in Exp. 1 (Table 1). Diets were analyzed for DM, GE, CP, Ca, P, NDF, ADF, and AA, and fecal samples were analyzed in duplicate for GE. The GE in urine was determined in triplicate samples after being lyophilized as previously outlined (Kim et al., 2009). Values for the ATTD of energy in each diet were calculated (Widmer et al., 2007). The amount of energy lost in the feces and in the urine, respectively, was calculated, and the quantities of DE and ME in each of the 4 diets were calculated (Widmer et al., 2007). The DE and ME in corn were calculated by dividing the DE and ME values for the corn diet by the inclusion rate of corn in the diet. These values were then used to calculate the contribution from corn to the DE and ME in the corn-SBM diets, and the DE and ME in each source of SBM was calculated by difference (Widmer et al., 2007). Data were analyzed using the Proc GLM procedure as outlined for Exp. 1.

RESULTS

The CP and AA concentrations were greater in HP-200, HP-310, and HP-340 than in SBM-CV (Table 1), but the concentrations of CP and AA were less in HP-200 than in HP-310 and HP-340. The concentrations of ADF and NDF were also greater in HP-200, HP-310, and HP-340 than in SBM-CV, but the concentrations

Table 3. Analyzed composition of experimental diets (as-fed basis), Exp. 1[1]

	No phytase			Phytase (500 units/kg)		
Item	HP-310	HP-340	SBM-CV	HP-310	HP-340	SBM-CV
DM, %	91.60	91.53	90.12	91.64	91.50	90.27
CP, %	22.19	21.37	21.48	21.48	21.74	20.46
Ca, %	0.37	0.38	0.44	0.44	0.37	0.46
P, %	0.31	0.29	0.32	0.32	0.30	0.33
Phytase, units/kg	85	130	<70	820	680	640
Indispensable AA, %						
Arg	1.63	1.55	1.52	1.57	1.54	1.51
His	0.57	0.57	0.56	0.55	0.56	0.54
Ile	1.09	1.04	0.99	1.07	1.03	1.00
Leu	1.82	1.73	1.66	1.76	1.71	1.66
Lys	1.42	1.41	1.34	1.37	1.40	1.33
Met	0.29	0.28	0.29	0.28	0.28	0.29
Phe	1.18	1.12	1.08	1.14	1.11	1.07
Thr	0.86	0.83	0.81	0.81	0.82	0.79
Trp	0.31	0.31	0.31	0.31	0.30	0.30
Val	1.11	1.08	1.02	1.10	1.07	1.04
Dispensable AA, %						
Ala	1.00	0.98	0.92	0.97	0.97	0.91
Asp	2.61	2.47	2.40	2.52	2.44	2.38
Cys	0.28	0.27	0.29	0.26	0.27	0.30
Glu	4.12	3.89	3.78	3.96	3.84	3.75
Gly	0.96	0.93	0.89	0.93	0.92	0.88
Pro	1.12	1.07	1.03	1.06	1.04	1.02
Ser	1.00	0.94	0.91	0.92	0.92	0.87
Tyr	0.66	0.65	0.67	0.65	0.67	0.68

[1]HP-310 (Hamlet Protein A/S, Horsens, Denmark) = enzyme-treated soybean meal; HP-340 = enzyme-treated soybean meal with phytase; and SBM-CV = conventional soybean meal that has not been enzyme treated.

Table 4. Ingredient composition of experimental diets (as-fed basis), Exp 2

	Diet[1]			
Ingredient, %	Corn	HP-200	HP-310	SBM-CV
Ground corn	96.45	68.00	68.00	62.00
HP-200	—	29.00	—	—
HP-310	—	—	29.00	—
SBM-CV	—	—	—	35.00
Ground limestone	1.35	0.9	0.9	0.9
Monocalcium phosphate	1.50	1.40	1.40	1.40
NaCl	0.40	0.40	0.40	0.40
Vitamin-micromineral premix[2]	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00

[1]HP-200 (Hamlet Protein A/S, Horsens, Denmark) = short-time enzyme-treated soybean meal; HP-310 = enzyme-treated soybean meal; and SBM-CV = conventional soybean meal that has not been enzyme treated.

[2]The vitamin-micromineral premix provided the following quantities of vitamins and microminerals per kilogram of complete diet: vitamin A, 11,128 IU; vitamin D_3, 2,204 IU; vitamin E, 66 IU; vitamin K, 1.42 mg; thiamin, 0.24 mg; riboflavin, 6.58 mg; pyridoxine, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid, 23.5 mg; niacin, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

of sucrose, raffinose, and stachyose were much greater in SBM-CV than in the enzyme-treated SBM. The trypsin inhibitor activity was low in all sources of SBM, but the concentration of β-conglycinin in SBM-CV was much greater than in the 3 enzyme-treated SBM. The concentration of glycinin was also much greater in SBM-CV than in the other 3 SBM, but HP-200 contained more glycinin than HP-310 and HP-340.

Table 5. Analyzed energy and nutrient composition of experimental diets (as-fed basis), Exp. 2

	Diet[1]			
Item	Corn	HP-200	HP-310	SBM-CV
DM, %	87.82	88.14	88.68	87.99
GE, kcal/kg	3,734	3,942	3,943	3,898
CP, %	8.22	21.23	21.52	20.87
Ca, %	0.76	0.66	0.67	0.72
P, %	0.54	0.62	0.65	0.67
NDF, %	8.99	9.65	12.69	9.62
ADF, %	2.03	3.12	3.09	2.94
Indispensable AA, %				
Arg	0.42	1.36	1.39	1.45
His	0.22	0.55	0.55	0.58
Ile	0.27	0.91	0.91	0.94
Leu	0.90	1.81	1.86	1.86
Lys	0.31	1.10	1.15	1.23
Met	0.15	0.31	0.30	0.33
Phe	0.38	1.03	1.05	1.07
Thr	0.28	0.76	0.78	0.80
Trp	0.10	0.27	0.27	0.29
Val	0.37	1.00	0.99	1.03
Dispensable AA, %				
Ala	0.55	1.05	1.06	1.07
Asp	0.58	2.10	2.14	2.22
Cys	0.16	0.31	0.29	0.33
Glu	1.47	3.64	3.75	3.80
Gly	0.33	0.86	0.86	0.89
Pro	0.61	1.12	1.17	1.19
Ser	0.35	0.87	0.91	0.90
Tyr	0.28	0.68	0.69	0.71

[1]HP-200 (Hamlet Protein A/S, Horsens, Denmark) = short-time enzyme-treated soybean meal; HP-310 = enzyme-treated soybean meal; and SBM-CV = conventional soybean meal that has not been enzyme treated.

P Digestibility (Exp. 1)

Feed intake, DMI, and the ATTD of DM were not different among diets (Table 6). However, the total fecal output of P was reduced ($P < 0.001$) when phytase was added to the diets and the P output from pigs fed the HP-340 diet without phytase was less ($P < 0.001$) than the P output from pigs fed the HP-310 and the SBM-CV diets without phytase. The output of P from pigs fed the HP-340 diet with phytase was also less ($P < 0.001$) than for pigs fed the HP-310 diet or the SBM-CV diet with phytase. Pigs fed the HP-310 and the SBM-CV diets with phytase also had greater ($P < 0.001$) ATTD of P than pigs fed these diets without phytase. However, when HP-340 was used, no difference in the ATTD between the diet without and with phytase was observed. The ATTD of P was also greater ($P < 0.001$) for HP-340 than for HP-310 and SBM-CV if no phytase was used, and if phytase was used, the ATTD of P in HP-340 was greater ($P < 0.001$) than in HP-310. The ATTD of Ca was also increased ($P < 0.001$) as phytase was added to the diets and SBM-CV had a greater ($P < 0.001$) ATTD of Ca than HP-310 and HP-340.

Energy Digestibility (Exp. 2)

No differences were observed in GE intake or in GE excreted in the feces among pigs fed the 4 experimental diets (Table 7). Pigs fed the corn diet, however, excreted less ($P = 0.03$) GE in the urine than pigs fed the 3 SBM-containing diets. The ATTD of GE was not different among diets, but the DE of the HP-200, HP-310, and the SBM-CV diets were greater ($P = 0.01$) than the corn diet. The ME in the 3 SBM-containing diets was also greater ($P = 0.03$) than in the corn diet, but no differences among the SBM diets were observed.

The DE of HP-310 was greater ($P = 0.01$) than SBM-CV, but the DE of HP-200 was not different from other 2 sources of SBM. The DE of all 3 SBM was, however, greater ($P = 0.01$) than corn. The ME of HP-200 and HP-310 were also greater ($P = 0.05$) than corn, but the ME of SBM-CV was not different from that of any of the other ingredients. When calculated on a DM basis, no differences in the DE among HP-200, HP-310, and SBM-CV were observed, but each of those values were greater ($P = 0.01$) than corn. However, the ME was not different among any of the ingredients when calculated on a DM basis.

DISCUSSION

Composition of Ingredients

The composition of the SBM-CV concurs with published values (NRC, 1998; Sauvant et al., 2004; Cervantes-Pahm and Stein, 2008). The reason for the reduced concentration of sucrose and oligosaccharides in the enzyme-treated SBM compared with SBM-CV is

Table 6. Apparent total tract digestibility (ATTD) of DM, Ca, and P by pigs fed 3 sources of soybean meal without or with exogenous phytase[1,2]

	Diet, 0 units/kg of phytase			Diet, 500 units/kg of phytase				*P*-value			
Item	HP-310	HP-340	SBM-CV	HP-310	HP-340	SBM-CV	SEM	SBM	Phytase	SBM × phytase
Total feed intake, g/d	1,003	1,006	1,054	1,032	1,016	1,032	84.5	0.16	0.69	0.33
DMI, g/d	919.2	921.4	950	946	930.2	931.8	77.2	0.62	0.65	0.36
Ca intake, g/d	3.7[b]	3.8[b]	4.6[a]	4.5[a]	3.8[b]	4.7[a]	0.3	<0.001	<0.001	<0.001
P intake, g/d	3.14	2.96	3.36	3.34	3.06	3.4	0.3	<0.001	0.02	0.37
DM in feces, %	91.0	93.4	92.1	93.0	93.9	91.7	0.7	0.04	0.22	0.25
Ca in feces, %	4.8[c]	3.7[bc]	3.3[ab]	4.8[c]	3.1[ab]	2.4[a]	0.4	<0.001	0.11	0.45
P in feces, %	3.5[a]	1.3[cd]	3.4[a]	2.0[bc]	1.1[d]	2.0[b]	0.1	<0.001	<0.001	<0.001
DM output, g/d	33.0	34.2	31.2	34.8	33.8	32.0	10.1	0.40	0.65	0.87
Ca output, g/d	1.7[b]	1.4[ab]	1.1[ab]	1.8[b]	1.1[ab]	0.8[a]	0.7	<0.001	0.20	0.50
P output, g/d	1.3[a]	0.5[bc]	1.2[a]	0.7[b]	0.4[c]	0.7[b]	0.3	<0.001	<0.001	0.01
ATTD of DM, %	96.4	96.3	96.7	96.3	96.4	96.6	0.2	0.19	0.78	0.85
ATTD of Ca, %	53.6[d]	63.8[cd]	75.6[ab]	60.9[cd]	70.0[bc]	82.2[a]	3.6	<0.001	0.03	0.99
ATTD of P, %	59.8[c]	83.8[ab]	65.5[c]	77.7[b]	87.7[a]	79.5[ab]	1.9	<0.001	<0.001	0.002

[a–d]Values within a row lacking a common superscript letter are different ($P < 0.05$).

[1]Each least squares mean represents 6 observations.

[2]HP-310 (Hamlet Protein A/S, Horsens, Denmark) = enzyme-treated soybean meal; HP-340 = enzyme-treated soybean meal with phytase; and SBM-CV = conventional soybean meal (SBM).

Table 7. Apparent total tract digestibility (ATTD) and concentration of DE and ME in diets and ingredients, Exp. 2[1]

	Diet[2]					
Item	Corn	HP-200	HP-310	SBM-CV	SEM	*P*-value
GE intake, kcal/d	2,843	3,092	3,141	3,074	195	0.74
GE fecal output, kcal/d	332	379	374	354	112	0.46
GE urine output, kcal/d	73[a]	135[b]	139[b]	130[b]	18	0.03
ATTD, %	88.4	87.6	88.0	88.5	0.5	0.55
DE in diets, kcal/kg	3,300[a]	3,454[b]	3,469[b]	3,448[b]	17	0.01
ME in diets, kcal/kg	3,207[a]	3,282[b]	3,297[b]	3,285[b]	24	0.03
DE in ingredients, kcal/kg	3,422[a]	3,887[bc]	3,940[c]	3,779[b]	57	0.01
ME in ingredients, kcal/kg	3,325[a]	3,522[b]	3,573[b]	3,460[ab]	64	0.05
DE in ingredients, kcal/kg of DM	3,891[a]	4,333[b]	4,316[b]	4,347[b]	63	0.01
ME in ingredients, kcal/kg of DM	3,780	3,926	3,914	3,980	71	0.22

[a–c]Values within a row lacking a common superscript letter are different ($P < 0.05$).

[1]Data are least squares means of 7 observations per treatment.

[2]HP-200 (Hamlet Protein A/S, Horsens, Denmark) = short-time enzyme-treated soybean meal; HP-310 = enzyme-treated soybean meal; and SBM-CV = conventional soybean meal.

most likely that sucrose and oligosaccharides are either fermented during the process of enzyme treatment or they are separated from the SBM after enzyme treatment. The total concentration of sucrose and oligosaccharides in SBM-CV is approximately 10.6%, and because of the disappearance of these carbohydrates in the enzyme-treated SBM, the concentration of the remaining nutrients increases. Therefore, the concentrations of CP, ether extract, ash, ADF, and NDF were greater in the enzyme-treated SBM than in SBM-CV. The concentration of glycinin and β-conglycinin was also much less in the enzyme-treated SBM than in SBM-CV, which indicates that enzyme treatment is effective in removing these antigens from SBM. The greater concentration of glycinin in HP-200 compared with HP-310 and HP-340 also indicates that the time of enzyme treatment is important for the removal of the antigens. The enzyme-treated SBM may, therefore, be better tolerated by weanling pigs than SBM-CV, and it may be possible to include greater concentrations of enzyme-treated SBM in diets fed to weanling pigs than if SBM-CV is used. The concentration of glycinin was less in HP-340 than in HP-200 and HP-310, but β-conglycinin concentration is the best predictor for the quantity of antigens in soybeans (Lalles et al., 1996) because β-conglycinin is more resistant to the proteolytic enzymes in the digestive processes than glycinin and other proteins (Zhao et al., 2008). As a consequence, β-conglycinin contributes a relatively greater proportion of antigens in SBM than does glycinin and there were no differences among the enzyme-treated SBM in the concentration of β-conglycinin.

P and Ca Digestibility

The ATTD of P in SBM-CV without phytase, which was measured in this experiment, was slightly greater than most published values (32 to 59%; Ajakaiye et al., 2003; Bohlke et al., 2005; Almeida and Stein, 2010). It is unknown why the ATTD of P in this particular source of SBM was greater than previously measured values, but the response to phytase observed in the present experiment was similar to the response obtained previously for SBM-CV (Almeida and Stein, 2010). Relatively large variability in the ATTD of P among sources of distillers dried grains with solubles has been reported (Pedersen et al., 2007). There are, however, very few published values for the ATTD of P in SBM, and to our knowledge, there are no data on the variability in the ATTD of P among sources of SBM. Nevertheless, the ATTD of P in SBM-CV measured in this experiment and in other experiments (Bohlke et al., 2005; Akinmusire and Adeola, 2009; Almeida and Stein, 2010) during the last decade indicate that the digestibility of P in SBM produced from current varieties of soybeans is much greater than values for the relative bioavailability of P that have been published (NRC, 1998). Values for the ATTD of P are expected to be greater than values for the relative bioavailability of P, but it is also possible that these differences are a result of changes in the soybeans being produced.

The fact that the ATTD of P in HP-310 was not different from the ATTD of P in SBM-CV indicates that the enzyme treatment used to produce HP-310 does not result in an increase in the ATTD of P. We are unaware of any other data for the ATTD of P in enzyme-treated SBM, but results of this experiment indicate that the ATTD of P in enzyme-treated SBM is similar to that of SBM-CV and the response to dietary phytase is similar for enzyme-treated SBM and SBM-CV.

The ATTD of P in HP-340 was much greater than the ATTD of P in HP-310 and in SBM-CV, which is most likely a result of the fact that microbial phytase was included in the enzyme mixture that was used to treat this source of SBM. It seems, therefore, that treatment of SBM with phytase is very effective in improving the ATTD of P. The present results also indicate that addition of microbial phytase to a diet containing

SBM that has already been treated with phytase has a much smaller effect in terms of increasing the ATTD of P than if phytase is added to a diet with a source of SBM that has not been treated with phytase. We are not aware of any previous experiments in which the effects of treating SBM with exogenous phytase have been reported, but results of the present experiment indicate that the procedure used to produce HP-340 was effective in improving the ATTD of P in SBM. It is, therefore, possible that this procedure may also be used to increase the ATTD of P in other ingredients.

All diets contained 0.70% limestone, and assuming that limestone contains 36% Ca (NRC, 1998), this amount of limestone contributed approximately 0.25% Ca to the diets, which is equivalent to about 70% of all the Ca in the diets with the remaining 30% coming from the SBM. The values for ATTD of Ca in the diets are, therefore, a combination of the ATTD of Ca in limestone and the ATTD of Ca in SBM. If the ATTD of Ca in SBM is assumed to be 46.7% (Bohlke et al., 2005) and the ATTD of Ca in limestone is between 75 and 80% (H. H. Stein, unpublished data), then the ATTD of the diets used in the present experiment should be approximately 65%, which is reasonably close to the values obtained. The ATTD of Ca in a diet containing 54% corn, 20% SBM, and 0.69% limestone is 62.3% (Stein et al., 2008), and the values for the ATTD of Ca obtained in this experiment also agree with this value. The increase in the ATTD of Ca that was observed when phytase was added to the diets is most likely a result of a reduced chelation of Ca to phytate as P is hydrolyzed from the phytate molecule (Selle et al., 2009). This observation is in agreement with previous reports (Guggenbuhl et al., 2007; Almeida and Stein, 2010).

The lack of any treatment differences on the output and ATTD of DM indicates that enzyme treatment of SBM does not affect DM digestibility even though the gross composition of the meals was changed. This observation is in agreement with data showing that addition of exogenous phytase to SBM does not affect DM digestibility (Nitrayová et al., 2009).

Energy Digestibility

The values for DE and ME in corn that were measured in this experiment are in close agreement with previous data (NRC, 1998; Sauvant et al., 2004; Baker and Stein, 2009). The DE and ME values for SBM-CV are slightly greater than the values reported by Sauvant et al. (2004), but close to other published values (NRC, 1998). The lack of a difference in ME between corn and SBM-CV is also in agreement with the data reported by Baker and Stein (2009). Results from the present experiment demonstrate that if expressed on a DM basis, there are no differences in the DE and ME between SBM-CV and enzyme-treated SBM despite the fact that almost all sucrose and oligosaccharides in the enzyme-treated SBM were removed during processing. The concentrations of ash, ADF, and NDF were, therefore, greater in the enzyme-treated SBM than in SBM-CV, but HP-200 and HP-310 also contained more CP and ether extract than SBM-CV, which is likely the reason that no differences in DE and ME among the SBM were observed. We are not aware of any other published data on the DE and ME of enzyme-treated SBM, but results from the present experiment demonstrate that enzymatic treatment of SBM does not reduce the DE and ME. As a consequence, values for DE and ME in SBM-CV may also be used for enzyme-treated SBM.

Conclusions

Enzymatic treatment of SBM changes the composition of the meals because sucrose, stachyose, and raffinose disappear during or after enzyme treatment. The concentrations of CP, ether extract, ash, ADF, and NDF are, therefore, greater in enzyme-treated SBM than in SBM-CV. However, these changes do not influence the digestibility of P and Ca, but a greatly improved ATTD of P is obtained if SBM is treated in the presence of microbial phytase. The concentration of DE and ME in the enzyme-treated SBM is not different from that of SBM-CV, but the concentration of DE in enzyme-treated SBM as well as in SBM-CV is greater than in corn.

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