

Effects of graded levels of microbial phytase on apparent total tract digestibility of calcium and phosphorus and standardized total tract digestibility of phosphorus in four sources of canola meal and in soybean meal fed to growing pigs

Y. She,*† Y. Liu,*¹ and H. H. Stein*‡²

*Department of Animal Sciences, University of Illinois, Urbana 61801; †Ministry of Agriculture Feed Industry Centre, State Key Laboratory of Animal Nutrition, China Agricultural University, Beijing 100193, P. R. China; and ‡Division of Nutritional Sciences, University of Illinois, Urbana 61801

ABSTRACT: One hundred twenty pigs were used to determine effects of graded levels of microbial phytase on the apparent total tract digestibility (ATTD) of P and Ca and the standardized total tract digestibility (STTD) of P in 4 sources of canola meal and in 1 source of soybean meal (SBM) fed to growing pigs. The 4 sources of canola meal were produced from 1 source of high-protein canola seeds and 2 sources of conventional canola seeds with 1 of the conventional canola seeds being divided into 2 separate batches before crushing. Pigs (16.2 ± 5.3 kg initial BW) were individually housed in metabolism crates and were randomly allotted to 1 of 20 diets in a 5×4 factorial arrangement of treatments with 5 ingredients and 4 levels of phytase. There were 6 replicate pigs per diet. Five basal diets based on high-protein canola meal (CM-HP), high-temperature processed canola meal (CM-HT), low-temperature processed canola meal (CM-LT), conventional canola meal (CM-CV), or SBM were formulated. The basal diets contained no phytase. Fifteen additional diets were prepared by adding approximately 500, 1,500, or 2,500 phytase units/kg to each of the 5 basal diets. Feces were quantitatively collected for 5 d based on the marker-to-marker approach after a 7-d adaptation period. Results

indicated that supplementation of microbial phytase increased (linear, $P < 0.05$) the ATTD of Ca in diets containing CM-HP, CM-HT, CM-CV, and SBM but not in diets containing CM-LT. Microbial phytase also increased (linear and quadratic, $P < 0.05$) the ATTD and STTD of P in all 5 ingredients. Compared with the CM-CV diets, the CM-HP diets had greater ($P < 0.05$) ATTD of Ca. The ATTD of Ca in the SBM diet was greater ($P < 0.05$) than in all canola meal diets, but no differences were observed in ATTD of Ca between CM-HT and CM-LT diets. The ATTD and the STTD of P were less ($P < 0.05$) in CM-HP, CM-HT, CM-LT, or CM-CV than in SBM if no microbial phytase was added, but no differences were observed in the ATTD and STTD of P in SBM, CM-HP, CM-HT, or CM-CV if the highest amount of phytase were added (interaction, $P < 0.05$). Regression equations were developed to calculate the response to microbial phytase on the STTD of P in CM-HP, CM-HT, CM-LT, CM-CV, and SBM. In conclusion, inclusion of graded levels of microbial phytase increased the ATTD and STTD of P in CM-HP, CM-HT, CM-LT, CM-CV, and SBM and the response to microbial phytase added to each ingredient can be predicted by regression equations.

Key words: calcium, canola meal, pigs, phosphorus, phytase, soybean meal

© 2017 American Society of Animal Science. All rights reserved. J. Anim. Sci. 2017.95:2061–2070
doi:10.2527/jas2016.1357

INTRODUCTION

Canola meal may be used as a source of digestible AA in diets for pigs (González-Vega and Stein, 2012; Cotten et al., 2016). Recently, new varieties of canola with greater concentrations of CP have been developed, and canola meal from these high-protein varieties (high-protein canola meal [CM-HP]) contains more CP and less NDF and ADF than

¹Present address: Department of Animal Science, University of California, Davis 95616.

²Corresponding author: hstein@illinois.edu

Received December 29, 2016.

Accepted February 27, 2017.

conventional canola meal (**CM-CV**; Liu et al., 2014; Berrocoso et al., 2015; Parr et al., 2015). Canola meal also provides significant quantities of P and Ca to diets (González-Vega et al., 2013; Rodríguez et al., 2013), but some Ca and more than 60% of total P in canola meal is bound to phytate (NRC, 2012). However, the digestibility of P in CM-HP has not been reported.

Different temperatures may be applied to canola meal during processing, and high-temperature processed canola meal (**CM-HT**) may have reduced phytic acid concentrations compared with canola meal processed at a lower temperature (low-temperature processed canola meal [**CM-LT**]; Satoh et al., 1998). It is, therefore possible that CM-HT has greater digestibility of Ca and P than CM-LT, but this hypothesis has not been experimentally verified.

Because of the relatively high concentration of phytic acid in canola meal, microbial phytase may improve Ca and P digestibility (Adeola and Cowieson, 2011; González-Vega et al., 2013; She et al., 2015). However, to our knowledge, effects of graded levels of microbial phytase on apparent total tract digestibility (**ATTD**) of Ca and P and standardized total tract digestibility (**STTD**) of P in CM-HP, CM-HT, or CM-LT have not been reported. Therefore, the objectives of this experiment were to determine effects of graded levels of microbial phytase on the ATTD of Ca and P and the STTD of P in CM-HP, CM-HT, and CM-LT and to compare these values with values obtained in CM-CV and soybean meal (**SBM**). The second objective was to develop regression equations to predict the response of microbial phytase on STTD of P in each of these ingredients.

MATERIALS AND METHODS

The protocol for the experiment was reviewed and approved by The Institutional Animal Care and Use Committee at the University of Illinois (Urbana-Champaign, IL). Pigs used in the experiment were the offspring of G-Performer boars and F-25 sows (Genetiporc Inc., Alexandria, MN).

Pigs, Diets, Feeding, and Sample Collecting

The ingredients used in this experiment were from the same batches as those used by Liu et al. (2014). The canola meals were produced from canola seeds that were grown within a narrow geographical area in British Columbia, Canada. One source of high-protein canola and 2 sources of conventional canola seeds were used. However, 1 of the 2 sources of conventional canola seeds was divided into 2 separate batches and processed at 2 different temperatures. Thus, a total of 4 different sources of canola meals were produced using the prepress solvent

Table 1. Composition of experimental diets, as-fed basis¹

Ingredient	Diet ²				
	CM-HP	CM-HT	CM-LT	CM-CV	SBM
CM-HP	22.50	–	–	–	–
CM-HT	–	30.00	–	–	–
CM-LT	–	–	29.00	–	–
CM-CV	–	–	–	27.00	–
SBM	–	–	–	–	47.00
Soybean oil	3.00	3.00	3.00	3.00	3.00
Cornstarch	63.27	55.82	56.80	58.85	38.92
Sucrose	10.00	10.00	10.00	10.00	10.00
Limestone	0.53	0.48	0.50	0.45	0.38
Salt	0.40	0.40	0.40	0.40	0.40
Vitamin–mineral premix ³	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00

¹For each ingredient, 3 additional diets were prepared by including microbial phytase (Optiphos 2000; Enzyvia, Sheridan, IN) at the expense of cornstarch to create diets containing 500, 1,500, or 2,500 phytase units/kg. Thus, a total of 20 diets were prepared with the 5 ingredients and 4 concentration of phytase.

²CM-HP = high-protein canola meal; CM-HT = high-temperature processed canola meal (processed at 99 to 105°C); CM-LT = low-temperature processed canola meal (processed at 91 to 95°C); CM-CV = conventional canola meal; SBM = soybean meal.

³Provided the following quantities of vitamins and microminerals per kilogram of complete diet: 11,128 IU vitamin A as retinyl acetate, 2,204 IU vitamin D₃ as cholecalciferol, 66 IU vitamin E as DL-alpha tocopheryl acetate, 1.42 mg vitamin K as menadiolone nicotinamide bisulfite, 0.24 mg thiamin as thiamine mononitrate, 6.58 mg riboflavin, 0.24 mg pyridoxine as pyridoxine hydrochloride, 0.03 mg vitamin B₁₂, 23.5 mg d-pantothenic acid as D-calcium pantothenate, 44 mg niacin as nicotinamide and nicotinic acid, 1.58 mg folic acid, 0.44 mg biotin, 10 mg Cu as copper sulfate, 125 mg Fe as iron sulfate, 1.26 mg I as potassium iodate, 60 mg Mn as manganese sulfate, 0.3 mg Se as sodium selenite, and 100 mg Zn as zinc oxide.

extraction process. The desolventizer–toaster temperature for producing CM-HP, CM-LT, and CM-CV was between 91 and 95°C, but the temperature used for producing CM-HT was between 99 and 105°C. During processing, the desolventizer–toaster temperatures were automatically monitored. The SBM was a commercial solvent extracted source of dehulled SBM that was obtained from Dupont Nutrition and Health, Solae LLC (Gibson City, IL).

A total of 120 growing barrows (16.2 ± 5.3 kg initial BW) were allotted to a randomized complete block design with 20 diets and 6 replicate pigs per diet. There were 3 blocks of 40 pigs and 2 replicates of each diet within each block. Pigs were housed in stainless steel metabolism crates (0.69 by 0.79 m) that were equipped with a feeder and a nipple drinker and fully slatted metal floors with diamond-shaped openings (1.5 by 4.0 cm). Below the slatted floor, a screen floor (1.4 by 1.4 mm openings) was installed to allow for total collection of fecal material from each pig.

Five diets were formulated using either CM-HP, CM-HT, CM-LT, CM-CV, or SBM as the sole source of P and AA, and these diets contained no microbial phytase (Tables 1 and 2). Three additional diets with

each ingredient were prepared by adding approximately 500, 1,500, or 2,500 phytase units (FTU) of microbial phytase (*Escherichia coli*; Optiphos 2000; Enzyvia, Sheridan, IN) per kilogram of diet, and the phytase was included at the expense of cornstarch. Therefore, a total of 20 diets were used. All diets were formulated to contain similar quantities of total P and Ca. Principles for diet formulations were similar to those previously used in experiments conducted to determine digestibility of P and effects of microbial phytase on P digestibility in canola meal (Rodríguez et al., 2013; Adhikari et al., 2015; Maison et al., 2015). However, because all P in the diets was provided by canola meal or SBM and canola meal contains more P than SBM, the inclusion of SBM was greater than the inclusion of canola meal. As a consequence, AA concentrations were different among diets and the concentration of some AA in the canola meal diets was less than what is required to maximize growth of pigs (NRC, 2012).

Diets were arranged in a 5 × 4 factorial design with 5 ingredients and 4 concentrations of microbial phytase for each ingredient. The quantity of feed provided per pig daily was calculated as 2.5 times the estimated requirement for maintenance energy (i.e., 197 kcal ME/kg^{0.60}; NRC, 2012) for the smallest pig in each replicate and divided into 2 equal meals that were provided at 0700 and 1600 h. Water was available at all times. The experiment lasted 12 d. The initial 7 d was considered an adaptation period to the diet, whereas feces were collected during the following 5 d according to 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 dried in a forced-air oven at 65°C for 5 d and ground through a 1-mm screen in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ).

Chemical Analyses

Feed ingredients were analyzed (Table 3) in duplicate for DM (method 927.05; AOAC International, 2007), ash (method 942.05; AOAC International, 2007), and CP (method 990.03; AOAC International, 2007). Acid hydrolyzed ether extract was determined by acid hydrolysis using 3 N HCl (Sanderson, 1986) followed by crude fat extraction using petroleum ether (method 2003.06; AOAC International, 2007) on a Soxtec 2050 automated analyzer (FOSS North America, Eden Prairie, MN). Ingredients were also analyzed in duplicate for GE using an adiabatic bomb calorimeter (model 6300; Parr Instrument Co., Moline, IL), for ADF (method 973.18; HAOAC International, 2007), for NDF (AOAC International, 2007), for lignin (method 973.18 [A–D]; AOAC International, 2007), and for sucrose, raffinose,

Table 2. Analyzed composition of diets, as-fed basis

Item	Diet ²																			
	CM-HP			CM-HT			CM-LT			CM-CV			SBM							
	Phytase, FTU ¹	500	1,000	1,500	Phytase, FTU	500	1,000	1,500	Phytase, FTU	500	1,000	1,500	Phytase, FTU	500	1,000	1,500				
DM, %	91.58	92.25	91.86	90.59	90.59	90.60	90.73	90.63	91.54	91.25	91.46	90.47	90.74	90.82	91.03	91.05	94.10	90.39	90.60	90.25
Ca, %	0.52	0.47	0.46	0.56	0.39	0.42	0.42	0.48	0.44	0.45	0.35	0.39	0.36	0.45	0.36	0.51	0.43	0.54	0.45	0.42
P, %	0.37	0.31	0.32	0.34	0.30	0.30	0.32	0.33	0.30	0.31	0.28	0.30	0.27	0.31	0.28	0.34	0.31	0.35	0.31	0.33
Phytase, FTU	<70	317	1,400	2,233	<70	547	1,633	2,833	113	697	1,633	2,167	<70	973	1,933	2,267	<70	597	1,333	2,133

¹Phytase expressed as phytase units (FTU) per kilogram of diet.

²CM-HP = high-protein canola meal; CM-HT = high-temperature processed canola meal (processed at 99 to 105°C); CM-LT = low-temperature processed canola meal (processed at 91 to 95°C); CM-CV = conventional canola meal; SBM = soybean meal.

Table 3. Analyzed composition of high-protein canola meal (CM-HP), high-temperature processed canola meal (CM-HT), low-temperature processed canola meal (CM-LT), conventional canola meal (CM-CV), and soybean meal (SBM), as-fed basis¹

Item	Ingredient				
	CM-HP	CM-HT	CM-LT	CM-CV	SBM
GE, kcal/kg	4,326	4,285	4,336	4,181	4,206
DM, %	90.22	88.44	90.35	88.63	87.55
CP (N × 6.25), %	44.72	36.02	36.99	34.20	47.11
Ash, %	7.85	6.51	6.74	8.03	6.48
AEE, ² %	3.32	3.65	3.25	3.39	1.87
NDF, %	20.81	28.06	26.98	30.91	8.34
ADF, %	13.84	18.96	19.20	18.76	4.72
Lignin, %	4.22	8.23	8.19	7.58	0.37
Sucrose, %	6.84	6.70	7.08	8.23	7.28
Raffinose, %	0.13	0.50	0.54	0.30	1.02
Stachyose, %	0.32	1.05	1.03	1.44	5.14
Fructose, %	ND ³	ND	ND	0.17	0.71
Glucose, %	0.10	ND	ND	ND	0.94
Ca, %	0.83	0.63	0.64	0.77	0.50
Total P, %	1.26	1.05	0.97	1.11	0.62
Phytate, %	4.06	2.90	2.98	3.13	1.58
Phytate P, ⁴ %	1.14	0.82	0.84	0.88	0.44
Nonphytate P, ⁵ %	0.12	0.23	0.13	0.23	0.18
Cr, mg/kg	2.10	2.40	3.30	0.50	<0.10
Co, mg/kg	1.58	<0.20	<0.20	<0.20	<0.20
Cu, mg/kg	7.0	5.7	5.8	5.6	13.9
Fe, mg/kg	100	149	162	236	113
Mg, %	0.64	0.59	0.60	0.56	0.30
Mn, mg/kg	54	61	62	60	36
Mo, mg/kg	1.3	0.9	0.9	0.7	3.0
K, %	1.53	1.17	1.20	1.34	2.30
Se, mg/kg	0.06	0.40	0.30	0.06	0.04
S, %	0.82	0.67	0.69	0.76	0.39
Na, mg/kg	29	38	35	1,180	35
Zn, mg/kg	53.2	51.7	52.0	67.9	43.3

¹The CM-HT was processed at a temperature between 99 and 105°C and CM-LT was processed at a temperature between 91 and 95°C.

²AEE = acid hydrolyzed ether extract.

³ND = nondetectable.

⁴Calculated as 28.2% of phytate (Sauvant et al., 2004).

⁵Calculated as the difference between phytate P and total P.

stachyose, fructose, and glucose (Janauer and Englmaier, 1978). Concentrations of Ca, P, Na, K, Mg, Co, Cu, Fe, Mn, Zn, Cr, Mo, and Se were analyzed in all ingredients by inductively coupled plasma optical emissions spectrometry (Optical Emission Spectrometer, model Optima 2000DV; PerkinElmer Inc., Waltham, MA) using an internally validated method (method 985.01; AOAC International, 2007) after wet ash sample preparation (method 975.03 B[b]; AOAC International, 2007). Ingredients were also analyzed for S (method 956.01; AOAC International, 2006), phytate (Ellis et al., 1977), and AA (method 982.30 E [a, b, c]; AOAC

International, 2007). Diet samples that were collected at the time of mixing and fecal samples were analyzed in duplicate for concentrations of DM, Ca, and P as described for the ingredients. Diets were also analyzed for phytase activity (Phytex Method, version 1; Eurofins Nutrition Analysis Center, Des Moines, IA).

Calculations and Statistical Analyses

The concentration of phytate P in each ingredient was calculated as 28.2% of phytate (Sauvant et al., 2004), and nonphytate P was calculated as the difference between the concentration of total P and phytate P. Values for ATTD of Ca and P and STTD of P were calculated in each diet (Almeida and Stein, 2010) using an assumed basal endogenous loss of 190 mg/kg DMI as suggested (NRC, 2012). Because canola meal or SBM was the only P-contributing ingredient in each diet, the ATTD and STTD of P also represent the ATTD and STTD values for each ingredient. Normality of data was confirmed and outliers were tested using the UNIVARIATE procedure in PROC MIXED in SAS (SAS Inst. Inc., Cary, NC). Data were analyzed by ANOVA using PROC MIXED. The model included diet, phytase, and the interaction between diet and phytase as fixed effects and replicate as a random effect. Least squares means were calculated using a least significant difference test, and means were separated using the PDIF statement and adjusted with the Bonferroni correction. Effects of adding graded levels of phytase to each ingredient were analyzed by linear and quadratic contrasts. Analyzed concentrations of phytase in the diets were used in these analyses, and coefficients for the unequally spaced concentrations of supplemental phytase were obtained using the IML procedure in SAS. Within each ingredient, regression equations to predict the responses of microbial phytase on the STTD of P were generated by PROC REG in SAS. Pig was the experimental unit for all calculations, and an α level of 0.05 was used to assess significance among means. If the *P*-value was between 0.05 and 0.10, responses were viewed as tendencies.

RESULTS

The analyzed activities of phytase in the CM-HP diets were <70, 317, 1,400, and 2,233 FTU/kg (Table 2). The analyzed activities of phytase in the CM-HT and CM-LT diets were <70, 547, 1,633, and 2,833 FTU/kg and 113, 697, 1,633, and 2,167 FTU/kg, respectively. The 4 CM-CV diets contained <70, 973, 1,933, and 2,267 FTU/kg of phytase and the 4 SBM diets contained <70, 597, 1,333, and 2,133 FTU/kg of phytase.

No differences in DMI were observed among treatments (Table 4). Inclusion of phytase did not affect Ca

Table 4. Effects of microbial phytase on apparent total tract digestibility (ATTD) of Ca and P and standardized total tract digestibility of P in diets containing high-protein canola meal (CM-HP), high-temperature processed canola meal (CM-HT), low-temperature processed canola meal (CM-LT), conventional canola meal (CM-CV), or soybean meal (SBM)¹

Item	Feed intake, g of DM/d	Ca intake, g/d	Ca in feces, g/d	P intake, g/d	P in feces, g/d	ATTD of Ca, %	ATTD of P, %	STTD of P, ³ %
CM-HP								
CM-HP + 0 FTU ² /kg	2,088	10.86 ^{ab}	4.05	7.72 ^a	4.37	62.95	43.65 ^f	48.78 ^f
CM-HP + 317 FTU/kg	2,152	10.12 ^{abcd}	2.69	6.67 ^{ab}	2.78	73.28	58.19 ^e	64.32 ^e
CM-HP + 1,400 FTU/kg	2,079	9.56 ^{abcde}	2.32	6.65 ^{ab}	2.17	75.95	67.46 ^{bcd}	73.40 ^{bcd}
CM-HP + 2,233 FTU/kg	2,062	11.55 ^a	2.37	7.01 ^{ab}	1.72	79.46	75.22 ^{abc}	80.81 ^{ab}
SEM	99.07	0.50	0.40	0.33	0.30	3.59	4.32	4.32
<i>P</i> -value for CM-HP								
Linear	0.659	0.368	0.004	0.301	<0.001	<0.001	<0.001	<0.001
Quadratic	0.859	0.011	0.043	0.069	0.005	0.155	0.004	0.002
CM-HT								
CM-HT + 0 FTU/kg	2,070	8.07 ^{cde}	3.55	6.21 ^{ab}	3.97	56.77	35.76 ^f	42.09 ^f
CM-HT + 547 FTU/kg	2,134	8.96 ^{bcd}	2.22	6.40 ^{ab}	2.43	74.96	61.76 ^{de}	68.10 ^{cde}
CM-HT + 1,633 FTU/kg	2,165	9.10 ^{bcd}	2.38	6.93 ^{ab}	2.14	73.82	69.14 ^{bcd}	75.08 ^{bcd}
CM-HT + 2,833 FTU/kg	2,100	10.08 ^{abcd}	1.96	6.93 ^{ab}	1.79	80.52	74.00 ^{abcd}	79.76 ^{abc}
SEM	107.07	0.46	0.29	0.33	0.20	2.82	2.67	2.67
<i>P</i> -value for CM-HT								
Linear	0.882	0.015	0.008	0.121	<0.001	<0.001	<0.001	<0.001
Quadratic	0.549	0.944	0.167	0.531	0.005	0.068	<0.001	<0.001
CM-LT								
CM-LT + 113 FTU/kg	2,188	9.63 ^{abcde}	3.33	6.57 ^{ab}	4.26	64.92	34.32 ^f	40.65 ^f
CM-LT + 697 FTU/kg	2,083	9.38 ^{abcde}	2.22	6.46 ^{ab}	2.10	76.59	67.68 ^{bcd}	73.81 ^{bcd}
CM-LT + 1,633 FTU/kg	2,190	7.66 ^c	2.19	6.13 ^{ab}	1.91	71.65	69.03 ^{bcd}	75.82 ^{bcd}
CM-LT + 2,167 FTU/kg	2,164	8.44 ^{cde}	2.41	6.49 ^{ab}	2.00	71.05	68.64 ^{bcd}	74.98 ^{bcd}
SEM	82.63	0.35	0.34	0.25	0.20	4.04	3.49	3.49
<i>P</i> -value for CM-LT								
Linear	0.875	0.004	0.116	0.598	<0.001	0.571	<0.001	<0.001
Quadratic	0.636	0.228	0.079	0.411	<0.001	0.160	<0.001	<0.001
CM-CV								
CM-CV + 0 FTU/kg	2,154	7.75 ^{de}	3.90	5.82 ^b	3.61	49.63	38.12 ^e	45.16 ^f
CM-CV + 973 FTU/kg	2,148	9.67 ^{abcde}	3.05	6.66 ^{ab}	2.44	67.62	63.52 ^{ab}	69.65 ^{bcd}
CM-CV + 1,933 FTU/kg	2,074	7.46 ^c	2.30	5.81 ^b	1.98	69.45	65.98 ^a	72.76 ^{bcd}
CM-CV + 2,267 FTU/kg	2,099	10.70 ^{abc}	2.78	7.14 ^{ab}	2.01	74.21	71.99 ^a	77.57 ^{abcd}
SEM	96.37	0.42	0.42	0.29	0.26	4.78	3.54	3.54
<i>P</i> -value for CM-CV								
Linear	0.550	0.011	0.019	0.067	<0.001	<0.001	<0.001	<0.001
Quadratic	0.935	0.934	0.382	0.931	0.111	0.278	0.006	0.006
SBM								
SBM + 0 FTU/kg	2,068	8.89 ^{bcd}	2.60	6.41 ^{ab}	2.60	70.73	59.54 ^{hi}	65.67 ^{de}
SBM + 597 FTU/kg	2,064	11.15 ^{ab}	2.00	7.22 ^{ab}	1.65	81.82	76.94 ^b	82.37 ^{ab}
SBM + 1,333 FTU/kg	2,081	9.37 ^{abcde}	1.14	6.45 ^{ab}	1.06	87.81	83.77 ^a	89.90 ^a
SBM + 2,133 FTU/kg	2,007	8.43 ^{cde}	1.22	6.62 ^{ab}	1.04	85.47	84.14 ^a	89.90 ^a
SEM	110.82	0.50	0.17	0.36	0.13	1.46	1.33	1.33
<i>P</i> -value for SBM								
Linear	0.728	0.119	<0.001	0.876	<0.001	<0.001	<0.001	<0.001
Quadratic	0.757	0.010	0.021	0.481	<0.001	<0.001	<0.001	<0.001
<i>P</i> -value								
Ingredient	0.647	<0.001	<0.001	0.029	<0.001	<0.001	<0.001	<0.001
Phytase	0.912	<0.001	<0.001	0.165	<0.001	<0.001	<0.001	<0.001
Ingredient × phytase interaction	1.000	<0.001	0.812	0.039	0.081	0.197	0.010	0.019

a–f,h,i Means within a column with no superscript in common are different.

¹The CM-HT was processed at a temperature between 99 and 105°C and CM-LT was processed at a temperature between 91 and 95°C.

²FTU = phytase units.

³STTD = standardized total tract digestibility. Values for STTD of P were calculated by correcting ATTD values for basal endogenous loss. The basal endogenous P loss was assumed to be 190 mg/kg DMI (NRC, 2012).

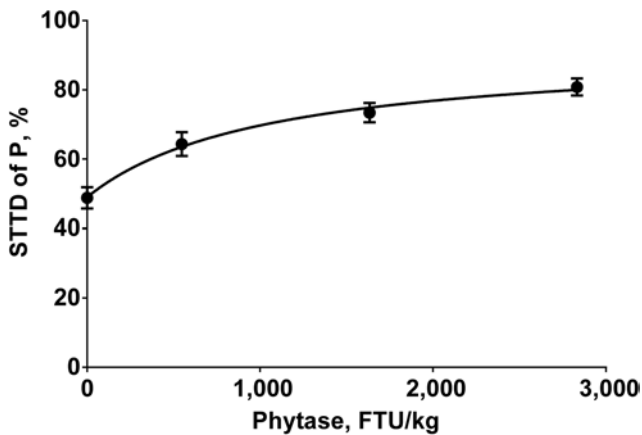


Figure 1. Regression equations for the effects of microbial phytase (phytase units [FTU]/kg) on the standardized total tract digestibility (STTD) of P in high-protein canola meal. Each observation (●) represents the average of 6 pigs per diet. The regression equation is as follows: $\text{STTD of P (\%)} = 50.47 + 0.022 \text{ FTU} - 0.00000396 \text{ FTU}^2$ ($P < 0.001$, adjusted $R^2 = 0.72$).

intake of pigs fed the CM-HP, CM-HT, or CM-LT diets, but phytase influenced ($P < 0.05$) the Ca intake of pigs fed the CM-CV and SBM diets (interaction, $P < 0.05$). Calcium output from pigs fed the SBM diets were less ($P < 0.05$) than from pigs fed the other diets, but there were no differences in Ca output among pigs fed the CM-HP, CM-HT, CM-LT, or CM-CV diets. Pigs fed the CM-HP diets had greater ($P < 0.05$) P intake than pigs fed the CM-CV diet without phytase, but no differences in P intake were observed among pigs fed the other diets (interaction, $P < 0.05$). The P output from SBM was less ($P < 0.05$) than from all canola meal diets. The ATTD of Ca in the SBM diets was greater ($P < 0.05$) than in all other diets, and the ATTD of Ca in CM-HP was greater ($P < 0.05$) than in CM-CV, but no difference in the ATTD of Ca between the CM-HT and CM-LT diets was observed. Pigs fed the SBM diet had greater ($P < 0.05$) ATTD and STTD of P than pigs fed all canola meal diets if no phytase was added to the diets. However, adding phytase to the diets improved ($P < 0.05$) the ATTD and STTD of P in the 5 ingredients to a different extent (interaction, $P < 0.05$), but no differences were observed for the ATTD and STTD of P among SBM, CM-HP, CM-HT, and CM-CV if the greatest dose of phytase was used.

Addition of microbial phytase to the diets decreased (linear, $P < 0.05$) Ca output and increased (linear, $P < 0.05$) the ATTD of Ca in the CM-HP, CM-HT, CM-CV, and SBM diets and tended to decrease (quadratic, $P = 0.079$) Ca output in the CM-LT diet. Microbial phytase also decreased (linear, $P < 0.05$) P output and increased (linear and quadratic, $P < 0.05$) the ATTD and STTD of P in pigs regardless of the ingredients included in the diets.

The quadratic response for the STTD of P to addition of increasing levels of microbial phytase to CM-HP is described by the following equation: STTD of P

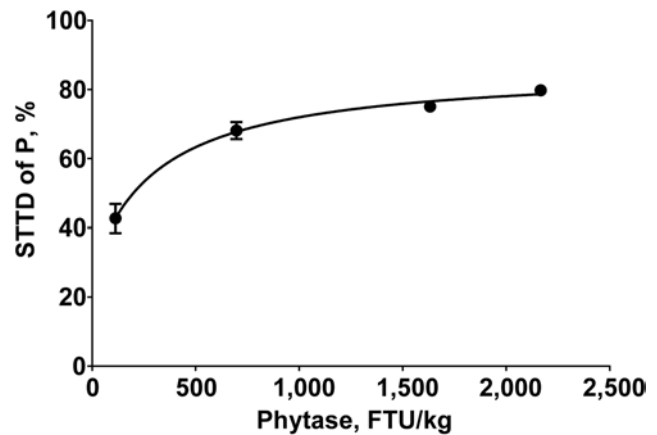


Figure 2. Regression equations for the effects of microbial phytase (phytase units [FTU]/kg) on the standardized total tract digestibility (STTD) of P in high-temperature (99 to 105°C) processed canola meal. Each observation (●) represents the average of 6 pigs per diet. The regression equation is as follows: $\text{STTD of P (\%)} = 39.53 + 0.044 \text{ FTU} - 0.0000122 \text{ FTU}^2$ ($P < 0.001$, adjusted $R^2 = 0.80$).

(%) = $50.47 + 0.022 \text{ FTU} - 0.00000396 \text{ FTU}^2$ ($R^2 = 0.72$; Fig. 1).

For CM-HT, the following equation was developed: $\text{STTD of P (\%)} = 39.53 + 0.044 \text{ FTU} - 0.0000122 \text{ FTU}^2$ ($R^2 = 0.80$; Fig. 2).

For CM-LT, the following equation was generated: $\text{STTD of P (\%)} = 42.01 + 0.045 \text{ FTU} - 0.0000136 \text{ FTU}^2$ ($R^2 = 0.77$; Fig. 3).

For CM-CV, the following equation was generated: $\text{STTD of P (\%)} = 46.69 + 0.035 \text{ FTU} - 0.00001003 \text{ FTU}^2$ ($R^2 = 0.73$; Fig. 4).

The following equation describes the response to phytase on the STTD of P in SBM: $\text{STTD of P (\%)} = 68.92 + 0.030 \text{ FTU} - 0.00000938 \text{ FTU}^2$ ($R^2 = 0.80$; Fig. 5).

These equations may be used to predict the STTD of P in CM-HP, CM-HT, CM-LT, CM-CV, and SBM with inclusion of any amount of phytase that is less than 2,233, 2,833, 2,167, 2,267, and 2,133 FTU/kg, respectively.

DISCUSSION

Composition of Ingredients

Canola is the registered name for rapeseed that is grown in North America and contains less than 2% of the total fatty acids in the oil as erucic acid and less than 30 μmol of alkenyl glucosinolates per gram of oil-free DM of the seed, and canola meal is the residual product that is left after oil is extracted from the seeds (Bell, 1993; Favero et al., 2014). Soybean meal is the major protein source used in diets for pigs (Stein et al., 2008), but the demand for SBM is rapidly increasing due to the continued increase in the global production of livestock and poultry, and as a consequence, possible alternative sources of protein for swine are needed

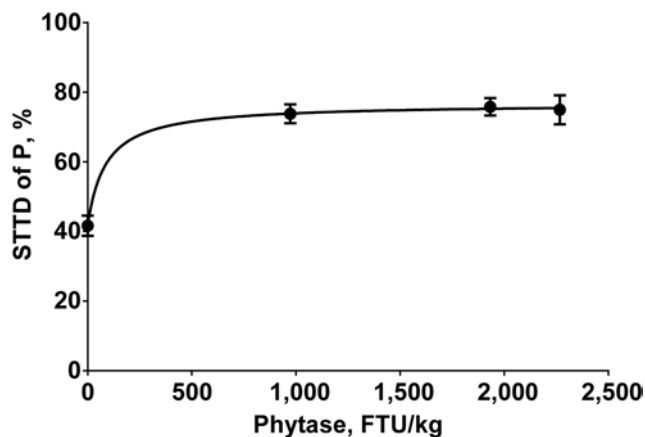


Figure 3. Regression equations for the effects of microbial phytase (phytase units [FTU]/kg) on the standardized total tract digestibility (STTD) of P in low-temperature (91 to 95°C) processed canola meal. Each observation (●) represents the average of 6 pigs per diet. The regression equation is as follows: $\text{STTD of P (\%)} = 42.01 + 0.045 \text{ FTU} - 0.0000136 \text{ FTU}^2$ ($P < 0.001$, adjusted $R^2 = 0.77$).

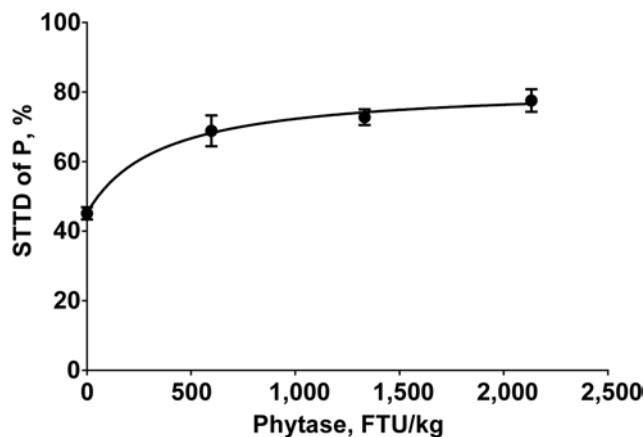


Figure 4. Regression equations for the effects of microbial phytase (phytase units [FTU]/kg) on the standardized total tract digestibility (STTD) of P in conventional canola meal. Each observation (●) represents the average of 6 pigs per diet. The regression equation is as follows: $\text{STTD of P (\%)} = 46.69 + 0.035 \text{ FTU} - 0.00001003 \text{ FTU}^2$ ($P < 0.001$, adjusted $R^2 = 0.73$).

(Cotten et al., 2016). Canola meal is the second most commonly used protein source for animal diets in the world (Arntfield and Hickling, 2011; Parr et al., 2015), but use of canola meal in diets for pigs is limited by the greater concentration of dietary fiber and reduced AA digestibility compared with SBM (Trindade Neto et al., 2012; Liu et al., 2014). However, new varieties of canola with larger seeds than conventional canola have been developed (Landerio et al., 2012; Liu et al., 2014; Sanjayan et al., 2014), and the concentration of CP in the meal from these varieties is increased and the concentration of NDF is reduced (Liu et al., 2014).

Analyzed concentrations of CP and AA in CM-CV and SBM used in this experiment were in agreement with reported values (González-Vega and Stein, 2012; NRC, 2012). As expected, CP was greater and the concentration of NDF and ADF was less in CM-HP than in CM-CV. The greater concentration of glucosinolates in CM-HP compared with CM-CV is in agreement with previous observations (Berrocoso et al., 2015; Parr et al., 2015). Concentrations of total and phytate P in CM-CV (Maison et al., 2015; NRC, 2012) and SBM (Dilger and Adeola, 2006; Goebel and Stein, 2011) were also in agreement with published values.

Effects of Graded Levels of Microbial Phytase on the Standardized Total Tract Digestibility of P

Phosphorus is the second most abundant mineral in the pig body (Viveros et al., 2002) and is involved in mineralization of bone tissue and metabolism of carbohydrates, fat, and nitrogenous compounds and is important in transmembrane transport of metabolites (Weremko et al., 1997). Canola meal and SBM have relatively high concentrations of P, and although most

of that P is bound to phytate, inclusion of microbial phytase will result in most of the P in canola meal and SBM becoming available for pigs (Rodríguez et al., 2013; Maison et al., 2015; Sotak-Peper et al., 2016). The STTD of P in CM-CV without phytase obtained in the current experiment is within the range of values observed in previous experiments (Rodríguez et al., 2013; Maison et al., 2015). Results of this experiment indicating that the STTD of P in canola meal is close to 80% if more than 2,000 FTU of phytase is used, which provides further evidence of the positive effects of phytase on the STTD of P in canola meal.

During conventional processing, canola seeds are preheated prior to flaking, flakes are then cooked to separate oil and protein, and initial oil removal takes place using a mechanical press (Unger, 2011). To remove additional oil, solvent extraction is used, and the resulting, deoiled meal is then desolventized using a toaster at a temperature that does not exceed 107 to 110°C. To avoid heat damage of the meal, some plants use lower temperatures in the desolventizing process, but the impact of lower temperatures on digestibility of P has not been reported. However, in distiller's dried grains with solubles, increased heating results in increased digestibility of P by broiler chickens (Amezcuca and Parsons, 2007), but we are not aware of any data demonstrating effects of processing temperatures on the STTD of P in canola meal. We therefore hypothesized that reduced temperatures used in canola processing may result in reduced digestibility of P, but the current results indicate that within the range of temperatures used in this experiment, no differences in STTD of P was observed.

Although the STTD of P in canola meal is less than in SBM, the greater concentration of total P in canola meal results in greater concentration of digestible P in canola meal than in SBM. Canola meal, therefore, contributes

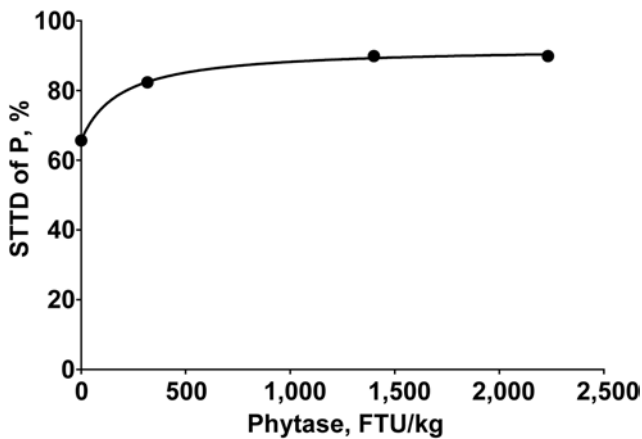


Figure 5. Regression equations for the effects of microbial phytase (phytase units [FTU]/kg) on the standardized total tract digestibility (STTD) of P in soybean meal. Each observation (●) represents the average of 6 pigs per diet. The regression equation is as follows: $\text{STTD of P (\%)} = 68.92 + 0.030 \text{ FTU} - 0.00000938 \text{ FTU}^2$ ($P < 0.001$, adjusted $R^2 = 0.80$).

a significant amount of digestible P to diets for pigs, and the need for supplementing P from feed phosphates is, therefore, reduced if canola meal rather than SBM is included in diets. As demonstrated in this experiment, if microbial phytase is included in the diets, the utilization of P from canola meal is relatively high, further increasing the amount of digestible P the pigs may obtain.

The concentration of phytate P in the canola meal used in this experiment was between 0.82 and 1.14%, which is within the range of reported values (NRC, 2012; Maison et al., 2015). The dose-dependent reduction in fecal P output as a result of inclusion of microbial phytase in the diets, regardless of the diet used, clearly indicates that microbial phytase is effective in hydrolyzing the phosphorus ester bond between P and phytate (Guggenbuhl et al., 2007; Augspurger et al., 2009), which results in increased absorption of P and increased STTD values for P.

To our knowledge, this is the first time the ATTD and STTD of P in CM-HP have been determined. However, the lack of any differences in the STTD of P between CM-HP and CM-CV demonstrates that the P in these 2 ingredients will be digested to the same degree, and this observation implies that digestibility values and responses to phytase obtained with CM-CV may be extrapolated to CM-HP.

The observation that the STTD of P in SBM without phytase was greater than in all the canola meals without phytase is a result of the greater percentage of P in canola meal that is bound to phytate compared with the P in SBM, as was illustrated by the chemical analysis of the ingredients. The fact that there was no difference in the STTD of P among SBM, CM-HP, CM-HT, and CM-CV at the greatest inclusion level of phytase further demonstrates that the main reason for the reduced STTD of P in canola meal without phytase is that most of the P is bound to phytate. The observation that microbial

phytase decreased P output and increased the STTD of P in pigs regardless of the ingredient is in agreement with previous data (Rodríguez et al., 2013; Rojas et al., 2013; Maison et al., 2015; Sotak-Peper et al., 2016) and confirms the effectiveness of microbial phytase in hydrolyzing the ester bond between phytate and P.

Effects of Graded Levels of Microbial Phytase on the Apparent Total Tract Digestibility of Ca

Phytate in plant feed ingredients may bind up to 6 atoms of Ca and promote the formation of Ca–phytate complexes, which reduces the ATTD of Ca in those ingredients (González-Vega et al., 2015). Canola meal has a relatively high concentration of Ca compared with other plant feed ingredients commonly used in diets for pigs (González-Vega et al., 2013). The increased ATTD of Ca and the reduced Ca excretion that was observed as microbial phytase was included in the diets demonstrates the effectiveness of phytase in hydrolyzing the Ca–phytate bond and is in agreement with previous data (González-Vega et al., 2013). The reduced ATTD of Ca in diets containing CM-HP or CM-CV compared with diets containing SBM is likely a result of the greater concentration of phytate in canola meal than in SBM.

Whereas all the P in all diets was furnished by canola meal or SBM, some of the Ca originated from the limestone that was added to the diets. The values for ATTD of Ca in the diets, therefore, represent the ATTD for a mixture of Ca from limestone and Ca from canola meal or SBM. However, Ca from limestone may bind to phytate from canola meal when the feed reaches the stomach of the animals, and microbial phytase, therefore, may increase the ATTD of Ca in limestone (González-Vega et al., 2015). As a consequence, the observed increase in ATTD of Ca as a result of addition of microbial phytase to the diets is likely a result of an increase in the ATTD of Ca from both canola meal and limestone.

Regression Equations

Regression equations were generated to predict responses of microbial phytase on the STTD of P in canola meal and SBM. The intercept value indicates the predicted STTD of P for the ingredient with no phytase. The observation that the quadratic terms for increased inclusion of phytase were significant for all ingredients indicates that addition of phytase to SBM or canola meal results in a linear-plateau response for the STTD of P, as is also indicated in Fig. 1 to 5. The reason for this response is most likely that as more bonds between P and phytate are hydrolyzed, less P can be released by additional phytase and at some point, no more P will

be released regardless of the quantity of phytase in the diet. A similar response was previously reported for addition of phytase to diets based on corn or corn germ (Almeida and Stein, 2012) and also for diets based on corn and SBM (Almeida et al., 2013). The regression equations obtained in this experiment for SBM, CM-CV, and CM-HP most likely are also representative for other sources of these ingredients, but validation of the equations with different sources of SBM, CM-CV, and CM-HP is needed before wider usage is recommended.

In conclusion, results of the present experiment demonstrate that the response to microbial phytase on the STTD of P in CM-HP canola meal is similar to that in CM-CV and that inclusion of approximately 1,500 FTU microbial phytase will result in STTD values of approximately 75% for both CM-HP and CM-CV. Inclusion of more than 2,000 FTU of phytase to CM-HP, CM-HT, or CM-CV will result in STTD values of P that are not different from the STTD of P in SBM, indicating that if enough phytase is added to canola meal, the bond between P and the inositol ring of phytate may be hydrolyzed. The regression equations that were developed from the results of this experiment may be used to predict the response to graded levels of phytase added to SBM, CM-HP, or CM-CV.

LITERATURE CITED

- Adeola, O. 2001. Digestion and balance techniques in pigs. In: A. J. Lewis and L. L. Southern, editors, Swine nutrition. CRC Press, Washington, DC. p. 903–916.
- Adeola, O., and A. J. Cowieson. 2011. Board-invited review: Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. *J. Anim. Sci.* 89:3189–3218. doi:10.2527/jas.2010-3715
- Adhikari, P. A., J. M. Heo, and C. M. Nyachoti. 2015. True and standardized total tract digestibility in canola meals from *Brassica napus* and *Brassica juncea* fed to growing pigs. *J. Anim. Sci.* 93:209–216. doi:10.2527/jas.2014-7569
- Almeida, F. N., and H. H. Stein. 2010. Performance and phosphorus balance of pigs fed diets formulated on the basis of values for standardized total tract digestibility of phosphorus. *J. Anim. Sci.* 88:2968–2977. doi:10.2527/jas.2009-2285
- Almeida, F. N., and H. H. Stein. 2012. Effects of graded levels of microbial phytase on the standardized total tract digestibility of phosphorus in corn and corn coproducts fed to pigs. *J. Anim. Sci.* 90:1262–1269. doi:10.2527/jas.2011-4144
- Almeida, F. N., R. C. Sulabo, and H. H. Stein. 2013. Effects of a novel bacterial phytase expressed in *Aspergillus oryzae* on digestibility of calcium and phosphorus in diets fed to weanling or growing pigs. *J. Anim. Sci. Biotechnol.* 4:8. doi:10.1186/2049-1891-4-8
- Amezcuca, C. M., and C. M. Parsons. 2007. Effect of increased heat processing and particle size on phosphorus bioavailability in corn distillers dried grains with solubles. *Poult. Sci.* 86:331–337. doi:10.1093/ps/86.2.331
- AOAC International. 2006. Official Methods of Analysis. 17th ed. W. Horwitz and G. W. Latimer, Jr., editors. AOAC International, Gaithersburg, MD.
- AOAC International. 2007. Official Methods of Analysis. 18th ed. W. Horwitz and G. W. Latimer, Jr., editors. AOAC International, Gaithersburg, MD.
- Arntfield, S., and D. Hickling. 2011. Meal nutrition and utilization. In: J. K. Daun, N. A. M. Eskin, and D. Hickling, editors, Canola: Chemistry, production, processing, and utilization. AOCS Press, Urbana, IL. p. 281–312. doi:10.1016/B978-0-9818936-5-5.50014-0
- Augspurger, N. R., J. D. Spencer, D. M. Webel, B. F. Wolter, and T. S. Torrance. 2009. An *Escherichia coli*-derived phytase can fully replace inorganic phosphorus in maize–soybean meal diets for growing-finishing pigs. *Anim. Feed Sci. Technol.* 154:254–259. doi:10.1016/j.anifeedsci.2009.08.013
- Bell, J. M. 1993. Factors affecting the nutritional values of canola meal: A review. *Can. J. Anim. Sci.* 73:689–697. doi:10.4141/cjas93-075
- Berrocoso, J. D., O. J. Rojas, Y. Liu, J. Shoulders, J. C. González-Vega, and H. H. Stein. 2015. Energy concentration and amino acid digestibility in high-protein canola meal, conventional canola meal, and soybean meal fed to growing pigs. *J. Anim. Sci.* 93:2208–2217. doi:10.2527/jas.2014-8528
- Cotten, B., D. Ragland, J. E. Thomson, and O. Adeola. 2016. Amino acid digestibility of plant protein feed ingredients for growing pigs. *J. Anim. Sci.* 94:1073–1082. doi:10.2527/jas.2015-9662
- Dilger, R. N., and O. Adeola. 2006. Estimation of true phosphorus digestibility and endogenous phosphorus loss in growing pigs fed conventional and low-phytate soybean meals. *J. Anim. Sci.* 84:627–634. doi:10.2527/2006.843627x
- Ellis, R., E. R. Morris, and C. Philpot. 1977. Quantitative determination of phytate in the presence of high inorganic phosphate. *Anal. Biochem.* 77:536–539. doi:10.1016/0003-2697(77)90269-X
- Favero, A., D. Ragland, S. L. Vieira, A. Owusu-Asiedu, and O. Adeola. 2014. Digestibility marker and ileal amino acid digestibility in phytase-supplemented soybean or canola meals for growing pigs. *J. Anim. Sci.* 92:5583–5592. doi:10.2527/jas.2014-7919
- Goebel, K. P., and H. H. Stein. 2011. Phosphorus digestibility and energy concentration of enzyme-treated and conventional soybean meal fed to weanling pigs. *J. Anim. Sci.* 89:764–772. doi:10.2527/jas.2010-3253
- González-Vega, J. C., and H. H. Stein. 2012. Amino acid digestibility in canola, cottonseed and sunflower products fed to finishing pigs. *J. Anim. Sci.* 90:4391–4400. doi:10.2527/jas.2011-4631
- González-Vega, J. C., C. L. Walk, Y. Liu, and H. H. Stein. 2013. Endogenous intestinal losses of calcium and true total tract digestibility of calcium in canola meal fed to growing pigs. *J. Anim. Sci.* 91:4807–4816. doi:10.2527/jas.2013-6410
- González-Vega, J. C., C. L. Walk, and H. H. Stein. 2015. Effects of microbial phytase on apparent and standardized total tract digestibility of calcium in calcium supplements fed to growing pigs. *J. Anim. Sci.* 93:2255–2264. doi:10.2527/jas.2014-8215
- Guggenbuhl, P., P. Quintana, and C. S. Nunes. 2007. Comparative effects of three phytases on phosphorus and calcium digestibility in the growing pig. *Livest. Sci.* 109:258–260. doi:10.1016/j.livsci.2007.01.109
- Janauer, G. A., and P. Englmaier. 1978. Multi-step time program for the rapid gas-liquid chromatography of carbohydrates. *J. Chromatogr. A* 153:539–542. doi:10.1016/S0021-9673(00)95518-3
- Landero, J. L., E. Beltranena, and R. T. Zijlstra. 2012. Growth performance and preference studies to evaluate solvent-extracted *Brassica napus* or *Brassica juncea* canola meal fed to weaned pig. *J. Anim. Sci.* 90:406–408. doi:10.2527/jas.53955

- Liu, Y., M. Song, T. Maison, and H. H. Stein. 2014. Effects of protein concentration and heat treatment on concentration of digestible and metabolizable energy and on amino acid digestibility in four sources of canola meal fed to growing pigs. *J. Anim. Sci.* 92:4466–4477. doi:10.2527/jas.2013-7433
- Maison, T., Y. Liu, and H. H. Stein. 2015. Apparent and standardized total tract digestibility by growing pigs of phosphorus in canola meal from North America and 00-rapeseed meal and 00-rapeseed expellers from Europe without and with microbial phytase. *J. Anim. Sci.* 93:3494–3502. doi:10.2527/jas.2015-9055
- NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.
- Parr, C. K., Y. Liu, C. M. Parsons, and H. H. Stein. 2015. Effects of high-protein or conventional canola meal on growth performance, organ weights, bone ash, and blood characteristics of weanling pigs. *J. Anim. Sci.* 93:2165–2173. doi:10.2527/jas.2014-8439
- Rodríguez, D. A., R. C. Sulabo, J. C. González-Vega, and H. H. Stein. 2013. Energy concentration and phosphorus digestibility in canola, cottonseed, and sunflower products fed to growing pigs. *Can. J. Anim. Sci.* 93:493–503. doi:10.4141/cjas2013-020
- Rojas, O. J., Y. Liu, and H. H. Stein. 2013. Phosphorus digestibility and concentration of digestible and metabolizable energy in corn, corn coproducts, and bakery meal fed to growing pigs. *J. Anim. Sci.* 91:5326–5335. doi:10.2527/jas.2013-6324
- Sanderson, P. 1986. A new method of analysis of feedingstuffs for the determination of crude oils and fats. In: W. Haresign and D. J. A. Cole, editors, Recent advances in animal nutrition. Butterworths, London, UK. p. 77–81. doi:10.1016/B978-0-407-01162-5.50009-5
- Sanjayan, N., J. M. Heo, and C. M. Nyachoti. 2014. Nutrient digestibility and growth performance of pigs fed diets with different levels of canola meal from *Brassica napus* black and *Brassica juncea* yellow. *J. Anim. Sci.* 92:3895–3905. doi:10.2527/jas.2013-7215
- Satoh, S., D. A. Higgs, B. S. Dosanjh, R. W. Hardy, J. Geoffery Eales, and G. Deacon. 1998. Effect of extrusion processing on the nutritive value of canola meal for chinook salmon (*Oncorhynchus tshawytscha*) in seawater. *Aquacult. Nutr.* 4:115–122. doi:10.1046/j.1365-2095.1998.00056.x
- Sauvant, D., J. Perez, and G. Tran. 2004. Tables of composition and nutritional value of feed materials. 2nd ed. Wageningen Academic Publishers, Wageningen, The Netherlands.
- She, Y., Y. B. Su, L. Liu, C. F. Huang, J. T. Li, P. Li, D. F. Li, and X. S. Piao. 2015. Effects of microbial phytase on coefficient of standardized total tract digestibility of phosphorus in growing pigs fed corn and corn co-products, wheat and wheat co-products and oilseed meals. *Anim. Feed Sci. Technol.* 208:132–144. doi:10.1016/j.anifeeds.2015.07.011
- Sotak-Peper, K. M., J. C. González-Vega, and H. H. Stein. 2016. Effects of production area and microbial phytase on the apparent and standardized total tract digestibility of phosphorus in soybean meal fed to growing pigs. *J. Anim. Sci.* 94:2397–2402. doi:10.2527/jas.2016-0353
- Stein, H. H., L. L. Berger, J. K. Drackley, G. F. Fahey Jr., D. C. Hernot, and C. M. Parsons. 2008. Nutritional properties and feeding values of soybeans and their coproducts. In: L. A. Johnson, P. J. White, and R. Galloway, editors, Soybeans: Chemistry, production, processing and utilization. AOCS Press, Urbana, IL. p. 613–660. doi:10.1016/B978-1-893997-64-6.50021-4
- Trindade Neto, M. A., F. O. Opepaju, B. A. Slominski, and C. M. Nyachoti. 2012. Ileal amino acid digestibility in canola meals from yellow- and black-seeded *Brassica napus* and *Brassica juncea* fed to growing pigs. *J. Anim. Sci.* 90:3477–3484. doi:10.2527/jas.2011-4773
- Unger, E. H. 2011. Processing. In: J. K. Daun, N. A. M. Eskin, and D. Hickling, editors, Canola: Chemistry, production, processing, and utilization. AOCS Press, Urbana, IL. p. 163–188. doi:10.1016/B978-0-9818936-5-5.50010-3
- Viveros, A., A. Brenes, I. Arija, and C. Centeno. 2002. Effects of microbial phytase supplementation on mineral utilization and serum enzyme activities in broiler chicks fed different levels of phosphorus. *Poult. Sci.* 81:1172–1183. doi:10.1093/ps/81.8.1172
- Weremko, D., H. Fandrejowski, and T. Zebrowska. 1997. Bioavailability of phosphorus in feeds of plant origin for pigs – Review. *Asian-Australas. J. Anim. Sci.* 10:551–566. doi:10.5713/ajas.1997.551