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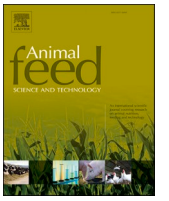

Standardized total tract digestibility of phosphorus in field peas fed to growing pigs is increased by microbial phytase, but particle size and origin of field peas do not affect digestibility of phosphorus

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ABSTRACT

Two experiments were conducted to test the hypothesis that particle size or origin of field peas does not influence apparent total tract digestibility (ATTD) or standardized total tract digestibility (STTD) of P, but that increasing levels of phytase will increase ATTD and STTD of P in field peas when fed to growing pigs. In experiment 1, one source of field peas was obtained from the U.S., and two sources were obtained from Canada (i.e., Canada 1 and Canada 2). The U.S. field peas were ground to 265, 457, or 678 μm, whereas the Canada 1 peas were ground to 253 μm, and the Canada 2 source was ground to 411 μm. The five batches of field peas were each included in one diet and fed to 50 growing pigs (16.36 ± 1.19 kg) with 10 replicate pigs per diet. In experiment 2, six diets based on the U.S. field peas ground to 678 μm were formulated to contain 0, 250, 500, 1000, 2000, or 4000 units per kg of microbial phytase and fed to 48 pigs (15.26 ± 0.91 kg) with eight replicate pigs per diet. In both experiments, field peas were the only source of P in the diets. Pigs were housed individually in metabolism crates and feces were collected for four days. Results of experiment 1 indicated that the ATTD and STTD of P were not affected by source of peas or by particle size of the field peas and it was concluded that growing location and variety do not influence STTD of P in field peas. Results of experiment 2 indicated that the ATTD of Ca and P and the STTD of P increased (linear, $P < 0.001$) as phytase increased in the diets, and fecal excretion of Ca and P was reduced as the concentration of dietary phytase increased (linear, $P < 0.001$). It was, therefore, concluded that if microbial phytase is included in diets containing field peas, the inclusion of feed phosphate can be reduced, and manure concentration of P will also be reduced. In conclusion, the hypotheses that neither growing location nor particle size influences STTD of P were confirmed, and the hypothesis that increased concentration of dietary phytase increases STTD of P was also confirmed.

Abbreviations: ATTD, apparent total tract digestibility; DM, dry matter; EPL, endogenous phosphorous losses; FTU, phytase units; STTD, standardized total tract digestibility.

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1. Introduction

The value of field peas as a source of high-quality protein and energy in diets for pigs has been demonstrated (Stein et al., 2004; 2006), and during the last 10 years, field pea production has increased in Canada and the U.S. by 60 and 20 %, respectively (FAO, 2022). However, the value of field peas in swine diets may be increased by using different processing techniques that maximize nutrient usage and reduce production costs (Stein and Bohlke, 2007; Lancheros et al., 2020). For example, the nutritional value of corn, soybean meal, and field peas fed to pigs may be improved by reducing the particle size (Montoya and Leterme, 2011; Rojas and Stein, 2015; 2017). However, the digestibility of P in corn and corn distillers dried grains with solubles is not increased by reducing the particle size of these ingredients (Liu et al., 2012; Rojas and Stein, 2015), but it is not known if the particle size influences the digestibility of P in field peas.

Phosphorus is one of the most expensive nutrients in diets for pigs, and much of the P in plants is bound to phytate, which is largely undigestible to pigs (Liao et al., 2005). Consequently, the digestibility of P in field peas is low (Stein et al., 2006; Lott et al., 2007; NRC, 2012), and feed phosphate needs to be added to the diet to provide sufficient P, which increases diet cost. Non-digested P excreted by pigs in feces may also have negative impacts on the external environment (Pizzeghello et al., 2011). Therefore, exogenous phytase is often included in diets for pigs because it increases the apparent total tract digestibility (ATTD) and the standardized total tract digestibility (STTD) of P in oilseed meals, legumes, and cereal grains by hydrolyzing the ester bond between P and the inositol ring in phytate (Stein et al., 2006; Almeida and Stein, 2010). Although values for digestibility of P in field peas without and with phytase have been reported (Helander et al., 1996; Stein et al., 2006), to our knowledge, there is no information about the impact on STTD of P of adding graded levels of microbial phytase to diets containing field peas. Likewise, there is no information about comparative values for digestibility of P in field peas grown in Canada and in the U.S., and it is not known if the STTD of P in field peas is affected by by growing location or variety of peas. Therefore, two experiments were conducted to test the hypotheses that 1) there are no differences in the STTD of P between field peas from Canada and peas from the U.S.; 2) particle size of field peas does not affect STTD of P; and 3) increasing levels of microbial phytase will increase STTD of P in field peas when fed to growing pigs.

2. Materials and methods

Two experiments were conducted, and protocols for both experiments were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois before animal work was initiated. Pigs used in both experiments were the offspring of Line 800 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN, USA).

2.1. Experimental diets and animals

2.1.1. Experiment 1: effect of origin and particle size of field peas

Three sources of field peas were used. One source was obtained from the U.S. (U.S. field peas), and two sources (CDC Meadow Yellow and CDC Amarillo Yellow) were obtained from Canada (i.e., Canada 1 and Canada 2). Field peas from the U.S. were ground using a hammer mill to a mean particle size of 265, 457, or 678 μm, whereas the Canada 1 source was only ground to 253 μm and the Canada 2 source was ground to 411 μm. Therefore, five batches of field peas were used in the experiment (Table 1). Field peas were the only P-contributing ingredient in the diets. Five diets containing each source of field peas in addition to sucrose and soybean oil were formulated (Table 2). Limestone was included in all diets to satisfy an overall Ca concentration of 3.5 g/kg. Vitamins, and minerals with the exception of Ca and P, were included in all diets to meet or exceed current nutritional requirement estimates for weanling pigs (NRC, 2012). Fifty growing pigs with an average initial body weight of 16.36 ± 1.19 kg were allotted to a randomized complete block

Table 1
Analyzed nutrient composition of five sources of field peas[a].

	Field pea particle size (μm)				
Item	678	457	411	265	253
Source:	U.S.	U.S.	Canada 2	U.S.	Canada 1
Dry matter, g/kg	891.4	892.1	897.2	895.4	899.9
Gross energy, MJ/kg	16.4	16.4	16.4	16.4	16.5
Crude protein, g/kg	198.5	196.3	200.3	199.0	195.2
Ash, g/kg	26.0	28.0	25.9	28.3	25.5
Acid-hydrolyzed ether extract, g/kg	9.5	9.5	10.3	9.3	10.0
Total P, g/kg	4.7	4.8	4.5	4.8	4.7
Phytate, g/kg	8.3	8.1	8.1	7.8	7.5
Phytate-bound P[b], g/kg	2.3	2.3	2.3	2.2	2.1
Non-phytate P[c], g/kg	2.4	2.5	2.2	2.6	2.6
Ca, g/kg	0.9	0.9	0.9	0.9	0.9
Potassium, g/kg	9.9	10.2	9.2	10.1	9.7
Magnesium, g/kg	1.3	1.2	1.3	1.3	1.4

[a] All values except dry matter are expressed on an 88 % dry matter basis. Peas were ground to a target particle size of 700, 450, or 250 μm.
[b] Phytate-bound P was calculated as 282 g/kg of P by phytic acid (Tran and Sauvant, 2004).
[c] Non-phytate P was calculated as the difference between total P and phytate-bound P.

Table 2
Ingredient and analyzed nutrient composition of experimental diets containing field peas, experiment [a].

Ingredient, g/kg	Field pea particle size (μm)				
	678	457	411	265	253
Source:	U.S.	U.S.	Canada 2	U.S.	Canada 1
Field peas	743.4	743.4	743.4	743.4	743.4
Soybean oil	40.0	40.0	40.0	40.0	40.0
Ground limestone	7.6	7.6	7.6	7.6	7.6
Sucrose	200.0	200.0	200.0	200.0	200.0
Sodium chloride	4.0	4.0	4.0	4.0	4.0
Vitamin-mineral premix[a]	5.0	5.0	5.0	5.0	5.0
Analyzed composition					
Dry matter	926.5	919.9	914.7	921.4	908.6
Ash	32.2	33.2	32.3	34.6	31.9
P	3.8	3.6	3.6	3.6	3.5
Ca	3.9	3.6	3.8	3.9	3.8

[a] The vitamin-mineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as cholecalciferol, 2210 IU; vitamin E as $_{DL}$-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; $_D$-pantothenic acid as $_D$-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxy chloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxy chloride.

design with five diets and two blocks of 25 pigs originating from two weaning groups. Within each block, the 25 pigs were randomly allotted to the five diets with five replicate pigs per diet in each block, resulting in a total of 10 replicate pigs per diet for the two blocks. The weaning group was the blocking factor.

2.1.2. Experiment 2: effect of microbial phytase on P digestibility

Six field peas-sucrose-based diets based on the U.S. field peas ground to 678 μm were formulated (Table 3). The six diets were identical, with the exception that they were formulated to contain 0, 250, 500, 1000, 2000, or 4000 units of microbial phytase (FTU; Quantum Blue; AB Vista, Marlborough, UK) per kg of diet. Field peas was the sole source of P in all diets. Limestone was included in the diets to satisfy an overall Ca concentration of 3.5 g/kg, and vitamins and minerals other than Ca and P were included in all diets to meet or exceed the estimated nutrient requirements for weanling pigs (NRC, 2012). A total of 48 growing pigs with an average initial body weight of 15.26 ± 0.91 kg were allotted to a randomized complete block design with six diets and three blocks of 12, 24, and 12 pigs each, and two replicate pigs per diet in the first and the third block and four replicate pigs per diet in the second block for a total of eight replicate pigs per diet. The three blocks contained pigs from three weaning groups that were weaned 14 days apart. The weaning group

Table 3
Ingredient and analyzed nutrient composition of experimental diets containing field peas, experiment [b].

	Phytase, units/kg diet					
Ingredient, g/kg	0	250	500	1000	2000	4000
Field peas	742.6	742.6	742.6	742.6	742.6	742.6
Soybean oil	40.0	40.0	40.0	40.0	40.0	40.0
Ground limestone	7.6	7.6	7.6	7.6	7.6	7.6
Sucrose	200.0	200.0	200.0	200.0	200.0	200.0
Sodium chloride	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin-mineral premix[a]	5.0	5.0	5.0	5.0	5.0	5.0
Phytase premix[b]	-	0.8	0.8	0.8	0.8	0.8
Corn starch	0.8	-	-	-	-	-
Analyzed composition						
Dry matter	929.2	926.2	926.7	928.4	923.8	940.0
Ash	30.4	31.4	31.2	31.1	31.1	30.7
P	3.2	3.0	3.0	3.0	3.0	3.3
Ca	3.5	3.6	3.5	3.7	3.6	3.5

[a] The vitamin-mineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as cholecalciferol, 2210 IU; vitamin E as $_{DL}$-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; $_D$-pantothenic acid as $_D$-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxy chloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxy chloride.

[b] The phytase premix was prepared by mixing corn starch and microbial phytase concentrate (QuantumBlue; AB Vista Feed Ingredients, Marlborough, UK; 5000 units of phytase/g). Five separate premixes were prepared by adjusting the inclusion of phytase in such a way that 0.8 g per kg of the phytase premix provided 250, 500, 1000, 2000, or 4000 phytase units per kg of complete diet.

was the blocking factor

2.2. Housing, feeding, and sample collection

In both experiments, pigs were housed individually in metabolism crates (0.71 × 0.84 m) that were equipped with a self-feeder, a nipple waterer, a fully slatted floor, and a screen floor to allow for total collection of fecal materials. The daily feed allowance was calculated as three times the estimated maintenance requirement for energy (i.e., 0.824 MJ metabolizable energy per kg body weight$^{0.60}$; NRC, 2012) and was provided each day in two equal meals at 0730 and 1400 hours. Addition of feed to each pig was recorded daily. Water was available at all times throughout the experiment. All pigs were fed experimental diets for 12 days, the initial five days of the experiment being the adaptation period to the diet, whereas fecal materials were collected from the feed provided during the following four days according to standard procedures for the marker-to-marker method (Adeola, 2001). Indigo carmine was used to mark the initiation of feces collection and was included in the morning meal on day 6. Fecal collection ceased when the second marker, ferric oxide, which was included in the morning meal on day 10, appeared in the feces. Orts were collected daily and weighed to determine feed intake from day six to day 10. During the collection period, feces were collected twice daily and stored at– 20 °C immediately after collection.

2.3. Chemical analysis

At the conclusion of each experiment, all fecal samples were thawed and then dried in a 65 °C forced air-drying oven (Metalab Equipment Corp., Hicksville, NY, USA) and finely ground using a 500 G stainless steel mill grinder (RRH, Zhejiang, China). Samples of field peas and diets were collected at the time of diet mixing. In both experiments, field peas, diets, and dried fecal materials were analyzed in duplicate for dry matter (DM) using oven drying at 135 °C for 2 hours (method 930.15; AOAC Int., 2019). Diets and field peas were also analyzed for dry ash at 600 ℃ (method 942.05; AOAC Int., 2019). Diets, field peas, and dried fecal materials were analyzed for Ca and P (method 985.01 A, B, and C; AOAC Int., 2019) using inductively coupled plasma-optical emission spectrometry (ICP-OES; Avio 200, PerkinElmer, Waltham, MA, USA). Sample preparation included dry ashing at 600 °C for 4 hours (method 942.05; AOAC Int., 2019) and wet digestion with nitric acid (method 3050 B; U.S. U.S. U.S. Environmental Protection Agency, 2000). Ingredient samples were analyzed for phytic acid (Ellis et al., 1977). Phytate-bound P in ingredients was calculated by multiplying the analyzed concentration of phytic acid by 0.282 of analyzed phytate (Tran and Sauvant, 2004), and non-phytate P was calculated by subtracting the amount of phytate P from total P. Ingredients were also analyzed for K and Mg using the same procedure as used to analyze Ca and P. Nitrogen in ingredients was determined by the combustion procedure using a LECO FP628 Nitrogen Analyzer (LECO Corp., St. Joseph, MI, USA; method 990.03; AOAC Int., 2019), and crude protein was calculated as analyzed N × 6.25. Gross energy in ingredient samples was measured using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA). Benzoic acid was used as the standard for calibration. Ingredients were also analyzed for acid-hydrolyzed ether extract using the acid hydrolysis filter bag technique (Ankom HCl Hydrolysis System; Ankom Technology, Macedon, NY, USA) followed by crude fat extraction using petroleum ether (AnkomXT15 Extractor; Ankom Technology, Macedon, NY, USA; method 2003.06, AOAC Int., 2019).

2.4. Calculations and statistical analysis

The ATTD of P in each diet was calculated using the direct procedure as described by Almeida and Stein (2010):

$$ATTD = (P_i - P_f)/P_i$$

where P_i is the total P intake (g) from day 6–10; and P_f is the fecal P excretion (g) in the feces originating from the feed that was provided from day 6–10. The same equation was used to calculate the ATTD of Ca and DM.

The STTD of P was calculated by correcting ATTD values for the basal endogenous phosphorus losses (EPL) using the following equation (NRC, 2012):

$$STTD = ATTD + EPL/\ P_i$$

where the EPL (g) from day 6–10 was assumed to be 190 mg per kg DM intake (NRC, 2012).

Data were analyzed using Proc MIXED of SAS (SAS Institute Inc.., 2016), and model assumptions on the residuals for both experiments were confirmed using the MIXED procedure and the Brown-Forsythe test of the GLM procedure of SAS. Outliers were detected using the ROBUSTREG procedure and were removed before final statistical analyses. Data for Ca and P digestibility were analyzed using the MIXED procedure of SAS, with the pig as the experimental unit. In experiment 1, the statistical model included the field pea batch as the main effect and block and replicate within block as random effects. Least-square means were calculated, and if the model was significant, means were separated using the PDIFF option with Tukey's adjustment. Polynomial contrast coefficients were also used to determine the linear effects of particle size within the U.S. source of peas on the digestibility of Ca and P and the interactive matrix language procedure in SAS (Proc IML) was used to generate appropriate coefficients for unequally spaced means. In experiment 2, the model included diet as the main effect, and polynomial contrast coefficients were used to determine linear and quadratic effects of phytase inclusion levels. Block and replicate within block were random effects. Results were considered significant at $P < 0.05$ and considered a tendency at $0.50 \leq P < 0.10$.

3. Results

For both experiments, all pigs consumed their diets throughout the experiment without apparent problems. The DM of field peas ranged from 891.4 to 899.9 g/kg, and ash ranged from 25.5 to 28.3 g/kg (Table 1). All field peas contained close to 0.9 g/kg Ca. The concentration of P was between 4.5 and 4.8 g/kg.

3.1. Experiment 1: effect of origin and particle size of field peas

Feed intake and weight of feces were not affected by source of field peas, but there was a tendency for a linear (P = 0.075) decrease in fecal excretion as particle size of the U.S. field peas was reduced (Table 4). A linear (P = 0.001) increase in the concentration of P in feces was observed when particle size of the U.S. peas was reduced. Pigs fed the U.S. peas ground to 457 μm or the Canada 2 source had greater (P < 0.05) absorption of P compared with pigs fed the Canada 1 source, but absorption of P by pigs fed diets containing the U.S. field peas was not affected by particle size. The ATTD and STTD of P were not affected by neither the source nor the particle size of field peas.

Daily intake of Ca was not different among diets (Table 5). Concentration of Ca in feces from pigs fed the U.S. peas ground to 678 μm and the U.S. peas ground to 457 μm or the Canada 2 peas was less (P < 0.05) than from pigs fed the U.S. peas ground to 265 μm, and concentration of Ca in feces increased linearly (P < 0.001) as the particle size of the U.S. field peas was reduced. Fecal Ca excretion increased linearly (P = 0.013) as the particle size of the U.S. field peas was reduced. Absorption of Ca was greater (P < 0.05) for Canada 2 peas than for the U.S. peas ground to 265 μm, and the ATTD of Ca was reduced (linear, P = 0.011) as the particle size of the U.S. peas was reduced.

3.2. Experiment 2: effect of microbial phytase on field peas

Feed intake and P intake tended to increase linearly (P = 0.077), whereas fecal excretion linearly (P = 0.001) decreased, as phytase increased in the diets (Table 6). Concentration of P in feces decreased (quadratic, P = 0.017), and P in feces expressed as g/day linearly (P < 0.001) decreased as phytase increased in the diets. In contrast, P absorption, and the ATTD and STTD of P as well as the ATTD of DM linearly (P < 0.01) increased by increasing phytase in the diets.

Calcium intake was not affected by the inclusion of phytase in the diets (Table 7). There was a quadratic (P = 0.005) decrease in the concentration of Ca in feces when phytase increased, and Ca excretion in feces expressed as g/day linearly (P < 0.001) decreased as phytase increased in the diets. However, Ca absorption and the ATTD of Ca linearly (P < 0.001) increased as phytase increased in diets.

4. Discussion

Analyzed concentration of Ca in the field peas used in these experiments agrees with reported values (NRC, 2012). However, the average P concentration in all sources of field peas was greater than the values reported by NRC (2012) and by Adekoya and Adeola (2022). Most P in plant-based ingredients is stored as phytic acid, and pigs lack endogenous phytase to degrade phytate; therefore, phytate-P is mostly unavailable for absorption (Cowieson et al., 2006; Woyengo and Nyachoti, 2013; Iyayi et al., 2013). The phytate-P concentration in the field peas used in the present experiments ranged from 2.1 g/kg to 2.3 g/kg, which was greater than reported values (NRC, 2012; Kahindi et al., 2015). Phytate-P concentration in feed ingredients may vary due to variations in P concentration among different varieties of the same ingredient, but values may also be influenced by the methods used to estimate phytate-P

Table 4
Effects of source of field peas and particle size on apparent total tract digestibility (ATTD) of dry matter (DM) and P and standardized total tract digestibility (STTD) of P, experiment 1[a].

Item	Field pea particle size (μm)					Field pea source		Contrast P-value, linear[b]
Particle size, μm:	678	457	411	265	253			
Source:	U.S.	U.S.	Canada 2	U.S.	Canada 1	SEM	P-value	Particle size
Feed intake, g/day	781	847	836	817	800	31.184	0.100	0.174
Fecal excretion, g/day	77.35	74.83	75.44	67.02	67.05	4.958	0.216	0.075
P intake, g/day	2.84^{ab}	3.08^{a}	3.00^{a}	2.97^{ab}	2.74^{b}	0.113	0.005	0.170
P in feces, g/kg	12.29	13.04	14.35	15.73	14.30	0.889	0.016	0.001
Fecal P excretion, g/day	1.03	1.04	1.07	1.14	1.07	0.110	0.742	0.221
P absorption, g/day	1.81^{ab}	2.03^{a}	1.93^{a}	1.83^{ab}	1.67^{b}	0.063	0.003	0.810
Basal EPL[c], mg/day	137	148	144	143	139	5.460	0.172	0.231
ATTD of DM	0.89^{b}	0.90^{ab}	0.90^{ab}	0.91^{a}	0.91^{a}	0.004	0.016	0.004
ATTD of P	0.64	0.65	0.62	0.62	0.61	0.030	0.608	0.544
STTD of P	0.69	0.70	0.67	0.67	0.66	0.030	0.644	0.539

[a-b]Within a row, means without a common superscript differ (P < 0.05).
[a] Data are least squares means of 10 observations per treatment.
[b] Linear contrast effect of particle size was determined among the U.S. sources.
[c] EPL= endogenous P loss. Values were calculated as basal EPL multiplied by daily DM intake. Basal EPL was estimated at 190 mg/kg DM intake (NRC, 2012).

Table 5
Effects of source of field peas and particle size on apparent total tract digestibility (ATTD) of Ca, experiment 1[a].

Item	Field pea particle size (μm)					Field pea source		Contrast P-value, linear[b]
Particle size, μm:	678	457	411	265	253			
Source:	U.S.	U.S.	Canada 2	U.S.	Canada 1	SEM	P-value	Particle size
Ca intake, g/day	2.88	3.04	3.12	3.02	3.06	0.135	0.297	0.209
Ca in feces, g/kg	10.71[b]	13.33[b]	11.97[b]	17.09[a]	14.30[ab]	1.221	<0.001	<0.001
Fecal Ca excretion, g/day	0.86	1.01	0.89	1.16	0.96	0.144	0.104	0.013
Ca absorption, g/day	2.03[ab]	2.03[ab]	2.22[a]	1.86[b]	2.10[ab]	0.071	0.016	0.094
ATTD of Ca[c]	0.71	0.67	0.71	0.62	0.69	0.035	0.049	0.011

[a-b]Within a row, means without a common superscript differ ($P < 0.05$).
[a] Data are least squares means of 10 observations per treatment, except for the diet containing the Canada 2 field peas ($n = 9$).
[b] Linear effect of particle size was determined among the U.S. sources.
[c] Although the model P-value was significant, none of the pairwise comparisons were significant.

Table 6
Effects of level of microbial phytase on the apparent total tract digestibility (ATTD) of dry matter (DM) and P and standardized total tract digestibility (STTD) of P in field peas, experiment 2[a].

Item	Phytase, units/kg diet							Contrast P-value	
	0	250	500	1000	2000	4000	SEM	Linear	Quadratic
Feed intake, g/day	735	733	760	749	761	793	47.615	0.077	0.618
Fecal excretion, g/day	67.86	59.86	62.44	61.44	52.33	47.14	4.249	0.001	0.402
P intake, g/day	2.67	2.67	2.76	2.72	2.77	2.89	0.169	0.077	0.618
P in feces, g/kg	13.85	10.76	10.44	8.87	7.49	7.51	0.513	<0.001	0.017
Fecal P excretion, g/day	0.92	0.64	0.65	0.54	0.43	0.36	0.047	<0.001	0.221
P absorption, g/day	1.67	2.04	2.12	2.13	2.35	2.54	0.180	<0.001	0.753
Basal EPL[b], mg/day	130	129	134	132	136	139	8.059	0.068	0.679
ATTD of DM	0.91	0.92	0.92	0.92	0.93	0.94	0.006	0.002	0.083
ATTD of P	0.68	0.78	0.76	0.78	0.84	0.87	0.022	<0.001	0.888
STTD of P	0.73	0.83	0.81	0.83	0.89	0.92	0.022	<0.001	0.883

[a] Data are least squares means of 8 observations per treatment, except for the diet containing no phytase ($n = 6$) and the diet containing 250 units of phytase ($n = 7$).
[b] EPL= endogenous P loss. Values were calculated as basal EPL multiplied by daily DM intake. Basal EPL was estimated at 190 mg/kg DM intake (NRC, 2012).

Table 7
Effects of phytase on the apparent total tract digestibility (ATTD) of Ca in diets containing field peas, experiment 2[a].

Item	Phytase, unit/kg diet							Contrast P-value	
	0	250	500	1000	2000	4000	SEM	Linear	Quadratic
Ca intake, g/day	2.62	2.71	2.66	2.60	2.70	2.81	0.167	0.236	0.386
Ca in feces, g/kg	9.65	7.29	5.98	6.60	5.59	5.36	0.456	<0.001	0.005
Fecal Ca excretion, g/day	0.64	0.45	0.42	0.36	0.31	0.22	0.029	<0.001	0.139
Ca absorption, g/day	1.98	2.26	2.23	2.20	2.38	2.55	0.173	<0.001	0.763
ATTD of Ca	0.75	0.84	0.84	0.86	0.88	0.92	0.014	<0.001	0.145

[a] Data are least squares means of 8 observations per treatment, except for the diets containing 0, 250, 1000, or 4000 units of phytase ($n = 7$).

concentration (Steiner et al., 2007). Nevertheless, in agreement with previous data (NRC, 2012), results from the current experiment demonstrated that less than 500 g/kg P in field peas is bound to phytate, which is less than in most other plant ingredients (Lee et al., 2023). The STTD of P in the field peas used in the current experiments was slightly greater than the STTD of P in field peas reported previously (Stein et al., 2006; NRC, 2012; Johnston et al., 2013), which may be a result of the lower proportion of phytate-bound P in the peas used in these experiments compared with peas used previously (Johnston et al., 2013).

Some of the variability in nutrient composition among feed ingredients may be related to differences in growing regions and the concentration and availability of minerals in the soil where they were grown (Uppström and Svensson, 1980; Lu et al., 2020). However, the observation that the different sources of field peas used in this experiment had minimum variation in the concentration of Ca and P indicates that there is no difference in concentrations of minerals between field peas grown in central Canada and peas grown in the northern region of the U.S. This observation is in agreement with data for soybean meal that demonstrated that growing location of soybeans had minimal impact on concentrations of minerals in soybean meal (Sotak-Peper et al., 2016). Likewise, the lack of a difference in STTD of P among the different sources of field peas used demonstrates that growing location did not influence digestibility of P in field peas. Previously, it was reported that growing location of canola, rapeseed, and soybeans did not influence STTD of P in these ingredients (Maison et al., 2015; Sotak-Peper et al., 2016). Therefore, the hypothesis that growing location of field peas did not

influence nutrient composition or STTD of P in field peas was confirmed.

Feed ingredients are ground to minimize particle size and improve nutrient and energy digestibility (Kim et al., 2002). The ATTD of gross energy and DM improved in sows and growing-finishing pigs when the particle size of cereal grains was reduced (Healy et al., 1994; Wondra et al., 1995). However, reduction of particle size did not change the standardized ileal digestibility of amino acids in corn or field peas when fed to growing pigs (Rojas and Stein, 2015; Ibagon et al., 2024). Likewise, reducing the particle size of corn from 865 to 339 μm did not affect ATTD or STTD of P (Rojas and Stein, 2015), and particle size reduction from 818 μm to 308 μm had no effect on the ATTD of P in corn distillers dried grains with solubles (Liu et al., 2012). The observation that particle size did not impact ATTD or STTD of P in field peas is, therefore, in agreement with results for other ingredients and indicates that liberation and absorption of P is not impaired by particle size. As a consequence, the hypothesis that particle size of field peas did not impact the STTD of P was confirmed.

When weanling pigs were fed coarse or fine-ground corn, the ATTD of Ca was not affected by particle size (Huang et al., 2015) and particle size of limestone did not impact STTD of Ca (Merriman and Stein, 2016). The observation in the current experiment that ATTD of Ca in field peas was reduced as particle size was reduced was, therefore, in contrast with results from other ingredients. Diets used in the current experiment contained almost 8 g/kg of limestone, and 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 field peas. It is possible that the reduced digestibility of Ca in diets with reduced particle size of peas is a consequence of increased precipitation in the intestinal tract because finer particles are expected to be more susceptible to precipitation, but research to confirm this hypothesis needs to be conducted.

Pigs do not produce sufficient endogenous phytase to hydrolyze the ester bond in the phytate molecule and liberate all bound P and chelated Ca (Liao et al., 2005), but inclusion of microbial phytase in diets for pigs results in hydrolysis of some of the ester bonds as the feed passes through the stomach, which results in release of P and Ca and increased P and Ca digestibility (Campbell and Bedford, 1992; Olsen et al., 2019). However, the amount of phytase required to optimize P digestibility may not be the same for all ingredients due to different amounts of phytate-bound P in the ingredients (Rojas and Stein, 2012; Almeida et al., 2017). An improvement of 0.10 in the ATTD and STTD of P in field peas upon inclusion of 675 FTU diet has been reported (Stein et al., 2006; Kahindi et al., 2015), and results of experiment 2 were, therefore, in agreement with previous data and also confirmed the hypothesis for the experiment.

The field peas with a particle size of 678 μm were arbitrarily chosen to be used for the phytase experiment, and because particle size did not impact STTD of P in experiment 1, it is unlikely that results for STTD of P in experiment 2 would have been different if peas with a different particle size had been used. The effectiveness of phytase may be impacted by the source of phytase and ingredient composition of the diet (Dias et al., 2010). The amount of P released by phytase is also highly correlated with the amount of phytase included in the diet, and a linear increase in the STTD of P in corn and corn co-products was observed as supplementation of phytase increased from 0 to 1100 FTU (Almeida and Stein, 2012). Likewise, the STTD of P in canola meal and soybean meal linearly increased when phytase addition increased from 0 to 2200 units (She et al., 2017), and the STTD of P in corn-soybean meal based diets also increased when dietary phytase increased from 0 to 4000 FTU per kg (Almeida et al., 2013; Espinosa et al., 2022; Lagos et al., 2022). Therefore, the observation that the ATTD and STTD of P in field peas increased linearly with the increase of phytase in diets is in agreement with data from experiments where phytase was added to diets containing other ingredients (Almeida and Stein, 2012; Dersjant-Li et al., 2017; Lagos et al., 2022). However, to the best of our knowledge, responses to graded levels of microbial phytase added to field peas have not been previously reported. The observation that the response to phytase was linear rather than quadratic indicates that before 4000 units of phytase were included in the diet, the source of phytase used in this experiment had the ability to remove P from phytate in a dose-dependent manner without reaching saturation. The fact that the STTD of P in the diet with the greatest inclusion of phytase was greater than 900 g/kg P indicates that the phytase used was able to liberate almost all P from the phytate molecule. This observation is in agreement with data for a corn-soybean meal diet where the inclusion of 4000 units of the same phytase resulted in STTD of P greater than 800 g/kg P (Lagos et al., 2022). The fact that the diets in experiment 2 had a concentration of P that was below the requirement is unlikely to have influenced the results for the STTD of P because feeding P below the requirement, at the requirement, or above the requirement for P does not influence total tract digestibility of P (Stein et al., 2008).

The observation that the ATTD of Ca increased as phytase was added to the diets is in agreement with previous data (Almeida et al., 2013; Gonzalez-Vega et al., 2015b) and reflects that endogenous Ca from ingredients may be bound to the phytate molecule. However, Ca from limestone can also be bound to phytate when it solubilizes in the stomach of pigs due to the negative charges on the phytate molecule, but if phytase is used, this binding is prevented and a greater ATTD of Ca is observed (Gonzalez-Vega et al., 2015a; Lee et al., 2019).

5. Conclusion

The hypotheses that neither the origin of field peas nor the particle size affect standardized total tract digestibility of P in field peas were confirmed, and field peas grown in Canada have standardized total tract digestibility of P of 0.67, which is not different from 0.68 of peas grown in the U.S. The hypothesis that increased concentration of microbial phytase linearly increases standardized total tract digestibility of P in field peas was also confirmed and a consequence of this observation is that fecal excretions of P and Ca will be reduced if phytase is added to diets containing field peas. Therefore, adding phytase to diets reduces the need for adding feed phosphates to the diets and fecal excretion of P and Ca will be reduced.

CRediT authorship contribution statement

Jimena A Ibagon: Writing – original draft, Investigation, Data curation. **Hans H Stein:** Writing – review & editing, Supervision,

Project administration, Methodology, Funding acquisition, Conceptualization. **Su A Lee:** Writing – review & editing, Supervision, Investigation, Data curation. **C. Martin Nyachoti:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors have no conflicts of interest.

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