



## Production region does not influence digestibility of calcium or phosphorus in sunflower co-products fed to growing pigs, but microbial phytase increases digestibility of both calcium and phosphorus

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### ABSTRACT

An experiment was conducted to test the hypothesis that the geographical location of production of sunflower co-products does not affect the apparent total tract digestibility (ATTD) of P and Ca and the standardized total tract digestibility (STTD) of P. Six sources of sunflower meal (SFM) were obtained from the U.S. (two sources), Ukraine (two sources), Hungary, and Italy. A source of sunflower expellers (SFE) from the U.S. was also used. Seven diets were formulated by mixing each source of sunflower co-product with cornstarch; therefore, the sunflower co-product was the only source of P in these diets. Seven additional diets that were identical to the previous seven diets, with the exception that 300 units per kg of microbial phytase were added to each diet, were also formulated. A total of 112 barrows (initial body weight:  $18.0 \pm 1.4$  kg) were allotted to the 14 diets using four blocks of 28 pigs, two pigs per diet in each block, and a total of eight replicate pigs per diet. Pigs were housed individually in metabolism crates to allow for the total collection of fecal materials for four days after seven days of adaptation to the diets. Diets and dried fecal samples were analyzed for dry matter, Ca, and P, and the ATTD of Ca and P, and STTD of P were calculated. Results indicated that diets containing phytase had greater ( $P < 0.001$ ) ATTD of Ca and P and greater ( $P < 0.001$ ) STTD of P than diets without phytase. Pigs fed the diet containing the SFE had reduced ( $P < 0.05$ ) ATTD and STTD of P compared with pigs fed diets containing SFM, but no differences in STTD of P among the six sources of SFM were observed. Pigs fed the diet containing one of the sources of SFM from the U.S. had greater ( $P < 0.05$ ) ATTD of Ca compared with pigs fed diets containing the Ukraine sources of SFM, but the ATTD of Ca was not different between SFM and SFE. In conclusion, there was no difference in the ATTD and STTD of P among SFM sources, but ATTD and STTD of P in SFE were less than in SFM. Small differences in the ATTD of Ca among SFM sources were calculated, but no difference between SFM and SFE was observed for ATTD of Ca. The ATTD and STTD of P and ATTD of Ca increased if microbial phytase was added to the diets containing SFM or SFE.

**Abbreviations:** ADFI, average daily feed intake; ATTD, apparent total tract digestibility; EPL, endogenous phosphorous losses; FTU, phytase units; SFE, Sunflower expellers; SFM, sunflower meal; STTD, standardized total tract digestibility.

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## 1. Introduction

Global oilseed production is increasing due to increased demand for oil for biodiesel and amino acids for feeding poultry and livestock (Goldsmith, 2008). Soybean meal is the premier source of amino acids for pigs and poultry (Stein et al., 2008), but sunflower co-products may be used as an alternative protein source for pigs. The majority of P in oilseed co-products is bound to phytate; however, pigs do not synthesize an adequate amount of endogenous phytase to liberate the P bound to phytate (Liao et al., 2005), and the digestibility of P in sunflower meal is, therefore, low (Rodríguez et al., 2013). Use of microbial phytase increases apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) of P in diets based on plant ingredients when fed to pigs due to the ability of phytase to hydrolyze the ester bond between P and phytate (Rojas and Stein, 2012; Almeida et al., 2013; Rodríguez et al., 2013). Values for ATTD of Ca and P and STTD of P in sunflower meal (SFM) without and with phytase have been reported (Rodríguez et al., 2013; Lee et al., 2021), but in each of these experiments, only one source of SFM was used. Likewise, in NRC (2012) only one source of SFM was cited for both the concentration and the digestibility of P, and no values for sunflower expellers (SFE) were available. There is, therefore, a need for a more comprehensive evaluation of digestibility of Ca and P in sunflower co-products. There are also no comparative values for the ATTD and STTD of P in sunflower co-products produced in different regions of the world and it is, therefore, not known if values for STTD of P or ATTD of Ca in SFM or SFE obtained in one region of the world are representative of sunflower co-products produced in other regions. Therefore, the objective of this experiment was to test the hypothesis that the ATTD and STTD of P in sunflower co-products of different origins are not different, but that regardless of the origin of the sunflower co-product, microbial phytase increases the digestibility of P and Ca in diets fed to growing pigs.

## 2. Materials and methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment. Pigs used in the experiment were the offspring of Line 359 boars and Camborough females (Pig Improvement Company, Hendersonville, TN, USA).

### 2.1. Animals, diets, and housing

A total of 112 barrows (initial body weight:  $18.0 \pm 1.4$  kg) were allotted to a randomized complete block design with 14 diets and four blocks of 28 pigs with two pigs per diet in each block for a total of eight replicate pigs per diet. The weaning date of pigs was the blocking factor. Seven sources of sunflower co-products were used. There were six sources of SFM (i.e., two sources from Ukraine, two sources from the U.S., one source from Hungary, and one source from Italy) and one source of sunflower expellers (SFE) from the U.S.

**Table 1**

Analyzed nutrient composition of six sources of sunflower meal (SFM) and one source of sunflower expellers (SFE)<sup>1,2</sup>.

Item	SFM						SFE			
	U.S. 1	U.S. 2	Ukraine 1	Ukraine 2	Hungary	Italy	Average	SD	CV	U.S.
Origin:	U.S. 1	U.S. 2	Ukraine 1	Ukraine 2	Hungary	Italy	Average	SD	CV	U.S.
Gross energy, MJ/kg	18.1	17.7	17.4	17.5	17.2	17.6	17.6	0.29	0.02	20.61
Dry matter g/kg	923.3	886.0	894.3	912.1	903.8	908.9	904.7	13.24	0.01	961.80
Crude protein, g/kg	326.2	273.4	366.3	367.5	330.0	324.5	331.3	34.53	0.10	268.70
Acid-hydrolyzed ether extract, g/kg	6.0	31.1	9.1	8.5	10.9	12.6	13.0	9.13	0.70	87.70
Insoluble dietary fiber, g/kg	323.1	415.2	286.3	298.1	309.6	359.2	331.9	47.92	0.14	368.70
Soluble dietary fiber, g/kg	41.0	40.7	37.4	5.8	27.3	98.8	41.8	30.91	0.74	41.20
Total dietary fiber, g/kg	365.0	455.9	323.7	302.9	335.9	458.0	373.6	67.65	0.18	409.90
Ash, g/kg	60.2	58.7	63.8	65.9	74.9	57.1	63.4	6.49	0.10	53.90
Ca, g/kg	3.2	3.5	3.8	4.0	4.1	4.7	3.9	0.52	0.13	3.00
Total P, g/kg	11.0	8.9	12.0	12.7	12.1	9.5	11.0	1.53	0.14	9.90
Phytic acid, g/kg	24.9	20.6	34.6	26.0	34.8	22.9	27.3	6.02	0.22	35.10
Phytate bound P <sup>3</sup> , g/kg	7.0	5.8	9.8	7.3	9.8	6.5	7.7	1.70	0.22	9.90
Non-phytate P <sup>4</sup> , g/kg	4.0	3.1	2.2	5.4	2.3	3.0	3.3	1.20	0.36	0.10
K, g/kg	15.5	15.0	14.5	15.1	13.6	13.2	14.5	0.91	0.06	11.90
Mg, g/kg	5.7	5.1	6.3	6.8	6.4	5.3	5.9	0.67	0.11	4.20
Na, mg/kg	140.3	1762.9	46.1	40.9	266.9	93.1	3917.3	6768.44	1.73	728.50
S, g/kg	37.1	466.9	313.7	264.6	270.0	441.2	298.9	154.43	0.52	389.70
Cu, mg/kg	32.4	37.4	41.2	38.7	39.9	32.7	370.8	37.20	0.10	234.50
Fe, mg/kg	102.7	99.6	110.5	102.3	491.7	176.2	1805.0	1552.33	0.86	824.70
Mn, mg/kg	42.8	36.3	45.8	47.1	51.7	34.4	430.2	66.28	0.15	285.50
Se, mg/kg	5.2	ND <sup>5</sup>	ND	ND	ND	ND	-	-	-	ND
Zn, g/kg	0.6	0.6	0.8	0.8	0.7	0.6	0.7	0.10	0.14	0.50

<sup>1</sup> All values except dry matter are expressed on an 880 g/kg dry matter basis.

<sup>2</sup> Values of gross energy, dry matter, ash, crude protein, acid hydrolyzed ether extract, and insoluble, soluble, and total dietary fiber had been adapted from Ibagón et al., (2021).

<sup>3</sup> Phytate-bound P was calculated as 282 g/kg of P by phytic acid (Tran and Sauvante, 2004).

<sup>4</sup> Non-phytate P was calculated as the difference between total P and phytate-bound P.

<sup>5</sup> ND = not detected.

(Table 1). Seven diets were formulated by mixing each source of sunflower co-product with sucrose and cornstarch, and the sunflower co-products were, therefore, the only source of P in the diets. Seven additional diets that were identical to the initial seven diets, with the exception that 300 units per kg of microbial phytase (FTU; Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK) were included in each diet were also formulated (Table 2). Limestone was included in all diets to satisfy an overall Ca concentration of 4.2 g/kg. Vitamins and minerals other than Ca and P were included in all diets to meet or exceed the estimated nutrient requirements for 11–25 kg pigs (NRC, 2012).

Pigs were placed in individual metabolism crates (0.71 × 0.84 m) equipped with a self-feeder, a nipple waterer, a fully slatted floor, and a screen floor that allowed for the total collection of fecal materials. All diets were fed in meal form. Pigs were limit-fed at three times the metabolizable energy requirement for maintenance (i.e., 0.824 MJ metabolizable energy per kg body weight<sup>0.60</sup>; NRC, 2012), which was provided each day in two equal meals at 0800 and 1600 h. Throughout the experiment, pigs had free access to water. Feed provisions were recorded daily, and all pigs were fed experimental diets for 14 days. The initial seven days of the experiment were considered the adaptation period to the diet, whereas fecal materials were collected from the feed provided during the following four days according to standard procedures using the marker-to-marker method (Adeola, 2001). Chromic oxide was used to mark the initiation of feces collection and was included in the morning meal on day eight. Fecal collection ceased when the second marker, ferric oxide, which was included in the morning meal on day 12, appeared in the feces. Orts were collected daily and weighed to determine feed intake from day eight to 12. During the collection period, feces were collected twice daily and stored at –20 °C immediately after collection.

## 2.2. Chemical analysis

At the conclusion of the experiment, all fecal samples were thawed and mixed within pig and diet and then dried in a 50 °C forced air-drying oven and finely ground using a 500 G stainless steel swing type mill grinder (RRH, Zhejiang, China) prior to analysis. Samples of the sunflower co-products and diets were collected at the time of diet mixing. Diets were analyzed for dry matter by oven drying at 135 °C for two hours (method 930.15; AOAC Int., 2019) and for dry ash at 600 °C (method 942.05; AOAC Int., 2019). Ingredients, diets, and fecal samples were also 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 four hours (method 942.05; AOAC Int., 2019) and wet digestion with nitric acid (method 3050 B; Environmental Protection Agency, 2000). Diets were analyzed for phytase activity (Phytex Method, Version 1; Eurofins, Des Moines, IA, USA), and ingredients were also 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; Lee et al., 2023), and non-phytate P was calculated by subtracting the amount of phytate P from total P. Ingredients were also analyzed for K, Mg, Na, Cu, Fe, Mn, S, Se, and Zn using the same procedure as to analyze Ca and P.

## 2.3. Calculations and statistical analyses

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

$$\text{ATTD} = (P_i - P_f)/P_i$$

where  $P_i$  is the total P intake (g) from day eight to 12; and  $P_f$  is the fecal P excretion (g) in the feces originating from the feed that was provided from day eight to 12. The same equation was used to calculate the ATTD of Ca.

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

**Table 2**

Composition of experimental diets containing sunflower meal (SFM) or sunflower expellers (SFE), as-fed basis.<sup>1</sup>

Item, g/kg	SFM						SFE
	U.S. 1	U.S. 2	Ukraine 1	Ukraine 2	Hungary	Italy	U.S.
Origin:							
Sunflower co-product	350	350	350	350	350	350	350
Corn starch	412	412	412	412	412	412	412
Soybean oil	20	20	20	20	20	20	20
Sucrose	200	200	200	200	200	200	200
Limestone	9	9	9	9	9	9	9
NaCl	4	4	4	4	4	4	4
Vitamin-mineral premix <sup>2</sup>	5	5	5	5	5	5	5

<sup>1</sup> Seven additional diets that were similar to the above diets with the exception that 300 units of phytase (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK) were added to these diets were also formulated.

<sup>2</sup> 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<sub>3</sub> 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<sub>12</sub>, 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.

$$\text{STTD} = \text{ATTD} + \text{EPL}/P_i$$

where the EPL (g) from day eight to 12 was calculated assuming 190 EPL mg per kg DM intake (NRC, 2012).

Data were analyzed using Proc MIXED of SAS, and model assumptions on the residuals were confirmed using the MIXED procedure and the Brown-Forsythe test of the GLM procedure of SAS (SAS Institute Inc., 2016). The MIXED procedure in SAS was used to generate studentized residuals to identify outliers. Observations with studentized residuals greater than 3 or less than  $-3$  were classified as outliers and removed prior to the final statistical analysis. One outlier was removed from the diet containing the U.S. source two SFM and another outlier was removed from the diet containing the Hungary source SFM. Data for the ATTD of Ca and P and STTD of P in the 14 diets were analyzed using PROC MIXED of SAS (SAS Institute Inc., 2016), with the experimental unit being the pig. The model included source of SFM, the level of phytase, and the interaction between source of SFM and phytase as fixed effects. Block and animal within block, were random effects. The least square means were calculated for each independent variable using the LSMeans statement in SAS (SAS Inst. Inc., Cary, NC, USA). If the model was significant, means were separated using the PDIF option with Tukey's adjustment. A second analysis was performed to compare SFE and SFM using a contrast statement, and the model used for this analysis included diet as fixed effect and block and animal within block as random effects. Results were considered significant at  $P < 0.05$  and considered a tendency at  $0.05 \leq P < 0.10$ .

### 3. Results

The concentration of Ca in SFM was between 3.2 and 4.7 g/kg, whereas SFE contained 3.0 g/kg Ca (Table 1). The concentrations of P in SFM was between 8.9 and 12.7 g/kg. Concentrations of K, Mg, and Zn were on average 14.5, 5.9, and 0.7 g/kg, respectively, for the six sources of SFM, whereas concentrations of K, Mg, and Zn in SFE were 11.9, 4.2, and 0.5 g/kg respectively.

Pigs remained healthy and readily consumed their diets throughout the experiment. The analyzed nutrient composition for all experimental diets was in agreement with the calculated values (Table 3). Neither the sample of sunflower co-product nor phytase influenced average daily feed intake or basal EPL (Table 4). There was no interaction between the sunflower source and inclusion of phytase for any parameters related to Ca and P intake, excretion, absorption, or digestibility. However, pigs fed the Ukraine 2 source without phytase had greater ( $P < 0.05$ ) intake of P, compared with pigs fed the other sources of SFM. When phytase was added to the diet, the concentration of P in feces was reduced ( $P < 0.001$ ) compared with diets without phytase, regardless of the source of SFM or SFE. Pigs fed diets containing the U.S. 2 source of SFM had reduced ( $P < 0.05$ ) concentration of P in feces compared with the Ukraine 2 source when phytase was added to the diets. When phytase was included in the diet, absorption of P was greater ( $P < 0.001$ ) than if no phytase was added to the diet, regardless of the sunflower co-product. Addition of phytase also increased ( $P < 0.001$ ) the ATTD and STTD of P in the sunflower co-products. There was no effect of phytase addition on daily EPL.

Inclusion of phytase in sunflower co-products based diets did not affect the daily Ca intake, but if phytase was not added to the diet, daily Ca intake was greater ( $P < 0.05$ ) in pigs fed the diet containing the SFM from Italy compared with pigs fed the diet containing the Ukraine 1 source of SFM (Table 5). Regardless of phytase addition, fecal Ca concentration was greater ( $P < 0.05$ ) for pigs fed the Ukraine 2 source than pigs fed the U.S. 2 source of SFM. When phytase was not added to the diets, the concentration of Ca in feces was

**Table 3**

Analyzed nutrient composition of experimental diets containing sunflower meal (SFM) or sunflower expeller (SFE) with or without phytase (FTU), as-fed basis.

Diets	Item, g/kg					
	Phytase, FTU/kg	Dry matter	Ash	Organic matter <sup>1</sup>	P	Ca
SFM U.S. 1	< 70	921.6	31.4	890.2	4.2	4.5
SFM U.S. 2	< 70	916.4	32.9	883.5	3.3	4.6
SFM Ukraine 1	< 70	915.1	33.8	881.4	4.6	4.5
SFM Ukraine 2	< 70	917.6	35.5	882.2	4.6	5.0
SFM Hungary	< 70	916.6	36.3	880.2	3.5	4.7
SFM Italy	< 70	918.4	39.3	879.1	4.9	4.8
SFE U.S.	< 70	926.1	30.7	895.4	3.6	5.2
SFM U.S. 1	250	919.5	37.5	882.0	4.1	4.7
SFM U.S. 2	230	917.7	33.2	884.5	3.4	4.9
SFM Ukraine 1	300	916.4	33.9	882.4	4.6	4.6
SFM Ukraine 2	300	917.8	35.7	882.0	4.8	5.1
SFM Hungary	330	895.6	36.7	858.9	3.4	4.8
SFM Italy	260	918.5	37.5	881.0	5.2	5.1
SFE U.S.	240	925.9	32.4	893.5	3.8	5.5

<sup>1</sup> Organic matter was calculated as dry matter–ash.

**Table 4**

Apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) of P in diets containing sunflower meal (SFM) and sunflower expellers (SFE) without or with 300 units of phytase.<sup>1</sup>

	ADFI <sup>2</sup> , g/d	P intake, g/d	P in feces, g/kg	Fecal P excretion, g/d	P absorption, g/d	Basal EPL <sup>3</sup> , mg/d	ATTD of P	STTD of P
No phytase								
SFM U.S. 1	0.87	3.59 <sup>de</sup>	13.60 <sup>abc</sup>	1.45 <sup>ab</sup>	2.14 <sup>bcd</sup>	151.56	0.59 <sup>ab</sup>	0.63 <sup>ab</sup>
SFM U.S. 2	0.86	2.89 <sup>f</sup>	10.99 <sup>bcd</sup>	1.32 <sup>ab</sup>	1.57 <sup>cd</sup>	150.40	0.55 <sup>ab</sup>	0.60 <sup>ab</sup>
SFM Ukraine 1	0.87	4.00 <sup>bcd</sup>	16.14 <sup>ab</sup>	1.58 <sup>ab</sup>	2.21 <sup>bcd</sup>	151.29	0.55 <sup>ab</sup>	0.65 <sup>ab</sup>
SFM Ukraine 2	0.92	4.63 <sup>a</sup>	17.86 <sup>a</sup>	1.75 <sup>a</sup>	2.69 <sup>ab</sup>	159.85	0.58 <sup>ab</sup>	0.66 <sup>ab</sup>
SFM Hungary	0.86	4.05 <sup>bcd</sup>	15.64 <sup>abc</sup>	1.64 <sup>a</sup>	2.41 <sup>abc</sup>	150.02	0.59 <sup>ab</sup>	0.63 <sup>ab</sup>
SFM Italy	0.86	3.20 <sup>ef</sup>	15.12 <sup>abc</sup>	1.68 <sup>a</sup>	1.52 <sup>d</sup>	150.74	0.47 <sup>b</sup>	0.52 <sup>b</sup>
Average	0.87	3.73	14.89	1.57	2.09	152.31	0.56	0.62
With phytase								
SFM U.S. 1	0.86	3.57 <sup>de</sup>	11.90 <sup>bcd</sup>	1.17 <sup>ab</sup>	2.39 <sup>abc</sup>	150.14	0.67 <sup>ab</sup>	0.72 <sup>a</sup>
SFM U.S. 2	0.87	2.93 <sup>f</sup>	7.47 <sup>d</sup>	0.88 <sup>b</sup>	2.05 <sup>bcd</sup>	152.48	0.70 <sup>a</sup>	0.75 <sup>a</sup>
SFM Ukraine 1	0.85	3.92 <sup>cd</sup>	12.13 <sup>abcd</sup>	1.15 <sup>ab</sup>	2.77 <sup>ab</sup>	148.54	0.71 <sup>a</sup>	0.74 <sup>a</sup>
SFM Ukraine 2	0.90	4.52 <sup>ab</sup>	14.61 <sup>abc</sup>	1.43 <sup>ab</sup>	3.09 <sup>a</sup>	156.19	0.69 <sup>a</sup>	0.72 <sup>a</sup>
SFM Hungary	0.90	4.21 <sup>abc</sup>	11.44 <sup>bcd</sup>	1.10 <sup>ab</sup>	3.11 <sup>a</sup>	152.39	0.74 <sup>a</sup>	0.79 <sup>a</sup>
SFM Italy	0.84	3.11 <sup>ef</sup>	10.10 <sup>cd</sup>	1.12 <sup>ab</sup>	1.99 <sup>bcd</sup>	146.58	0.64 <sup>ab</sup>	0.68 <sup>ab</sup>
Average	0.87	3.71	11.28	1.14	2.57	151.05	0.69	0.73
SEM	0.028	0.119	1.212	0.153	0.178	4.906	0.045	0.041
P-value								
SFM source	0.477	< 0.001	< 0.001	0.079	< 0.001	0.487	0.226	0.149
Phytase	0.811	0.805	< 0.001	< 0.001	< 0.001	0.658	< 0.001	< 0.001
SFE								
Without phytase	0.90	3.09	14.71	1.80	1.29	157.70	0.42	0.47
With phytase	0.90	3.11	9.00	1.12	1.99	158.39	0.65	0.70
P-value	0.927	0.927	0.028	0.033	0.001	0.931	0.002	0.002
SFM vs. SFE								
SEM	0.134	0.549	5.351	0.714	0.804	23.333	0.206	0.192
P-value	0.236	< 0.001	0.170	0.401	< 0.001	0.105	0.010	0.006

a-f Means within a row that do not have a common superscript tend to differ ( $P < 0.05$ ).

<sup>1</sup> Data are least squares means of eight observations per treatment. Except for the diets containing the Hungary and U.S. source 2, which had seven observations per treatment ( $n = 7$ ). Phytase  $\times$  SFM source interactions were not significant; therefore, they were not indicated.

<sup>2</sup> ADFI = average daily feed intake.

<sup>3</sup> EPL = endogenous P loss. Values were calculated as basal EPL multiplied by daily dry matter intake. Basal EPL was estimated at 190 mg/kg dry matter intake (NRC, 2012).

greater ( $P < 0.05$ ) for pigs fed the Ukraine 1 source compared with pigs fed diets containing the U.S. 1 source or the Italian SFM. However, pigs fed the SFM from Italy with phytase had a fecal Ca concentration that was not different from that of pigs fed one of the U.S. sources of SFM. Pigs fed the diet containing the Ukraine 2 source of SFM without phytase had greater ( $P < 0.05$ ) daily fecal Ca excretion compared with pigs fed the U.S. 2 source of SFM. Daily fecal excretion of Ca in feces was reduced ( $P < 0.001$ ) if phytase was added to the diet regardless of the source of SFM. When phytase was not added to the diets, pigs fed the SFM from Italy or the U.S. 2 source absorbed more ( $P < 0.05$ ) Ca than pigs fed the Ukraine 1 source of SFM. When phytase was added to the diet, pigs fed the U.S. 2 source of SFM had greater ( $P < 0.05$ ) absorption of Ca than pigs fed the Ukraine 1 source. Likewise, pigs fed the U.S. 1 source, the Ukraine 2 source, or the SFM from Hungary, or Italy had Ca absorption that was not different from that of pigs fed the U.S. 2 source of SFM. When phytase was not added to the diet, pigs fed diets containing the U.S. 2 source of SFM had greater ( $P < 0.05$ ) ATTD of Ca compared with pigs fed the diets containing one of the Ukraine sources of SFM. Pigs fed the diet containing the U.S. 2 source of SFM with phytase had greater ( $P < 0.05$ ) ATTD of Ca compared with pigs fed phytase-containing diets with one of the Ukraine sources. Diets containing phytase had greater ( $P < 0.001$ ) ATTD of Ca than diets without phytase, regardless of the source of SFM or SFE.

#### 4. Discussion

Sunflower co-products may be used as an alternative source of protein in diets for pigs due to its high concentration of protein and the absence of most anti-nutritional factors (Wahlstrom, 1992; Liu et al., 2015). Differences in the oil extraction process may affect the nutritional composition of sunflower co-products due to differences in the de-hulling process and the degumming process of the oil, which may affect the content of fat and fiber in the final co-product (Ibagón et al., 2021). Variations in the concentration of minerals in most oilseed co-products (including sunflower seeds) are influenced by cultivation methods, soil, and climate conditions (Jocić et al., 2015; Kolláthová et al., 2019).

Calcium and P are essential minerals involved in many physiological functions in pigs (Schröder et al., 1996; Crenshaw, 2001). However, because there is an interaction between Ca and P that can affect absorption of both minerals, inclusion of each mineral in pig diets must be carefully monitored. The concentration of P and Ca in de-hulled soybean meal is approximately 7.1 g/kg and 3.1 g/kg, respectively (NRC, 2012), but there is usually slightly more Ca and P in de-hulled sunflower co-products than in de-hulled soybean

**Table 5**Apparent total tract digestibility (ATTD) of Ca in diets containing sunflower meal (SFM) or sunflower expellers (SFE) without or with phytase.<sup>1</sup>

	Ca intake, g/d	Ca in feces, g/kg	Fecal Ca excretion, g/d	Ca absorption, g/d	ATTD of Ca
No phytase					
SFM U.S. 1	3.98 <sup>abc</sup>	16.17 <sup>bcd</sup>	1.73 <sup>abcd</sup>	2.25 <sup>cde</sup>	0.56 <sup>bcd</sup>
SFM U.S. 2	4.10 <sup>abc</sup>	12.07 <sup>de</sup>	1.43 <sup>bcd</sup>	2.68 <sup>abcd</sup>	0.66 <sup>abc</sup>
SFM Ukraine 1	3.96 <sup>bc</sup>	23.15 <sup>a</sup>	2.27 <sup>ab</sup>	1.69 <sup>e</sup>	0.43 <sup>d</sup>
SFM Ukraine 2	4.53 <sup>ab</sup>	26.34 <sup>a</sup>	2.54 <sup>a</sup>	2.00 <sup>de</sup>	0.44 <sup>d</sup>
SFM Hungary	4.35 <sup>abc</sup>	21.24 <sup>ab</sup>	2.22 <sup>ab</sup>	2.14 <sup>de</sup>	0.49 <sup>cd</sup>
SFM Italy	4.63 <sup>a</sup>	16.03 <sup>bcd</sup>	1.83 <sup>abcd</sup>	2.80 <sup>abcd</sup>	0.60 <sup>bcd</sup>
Average	4.26	19.17	2.00	2.26	0.53
With phytase					
SFM U.S. 1	3.95 <sup>bc</sup>	11.48 <sup>de</sup>	1.13 <sup>de</sup>	2.83 <sup>abcd</sup>	0.71 <sup>ab</sup>
SFM U.S. 2	4.15 <sup>abc</sup>	7.23 <sup>e</sup>	0.85 <sup>e</sup>	3.31 <sup>a</sup>	0.80 <sup>a</sup>
SFM Ukraine 1	3.88 <sup>c</sup>	16.21 <sup>bcd</sup>	1.54 <sup>bcd</sup>	2.34 <sup>bcd</sup>	0.60 <sup>bcd</sup>
SFM Ukraine 2	4.43 <sup>abc</sup>	20.41 <sup>abc</sup>	1.98 <sup>abc</sup>	2.46 <sup>abcd</sup>	0.55 <sup>bcd</sup>
SFM Hungary	4.52 <sup>abc</sup>	14.54 <sup>cd</sup>	1.46 <sup>bcd</sup>	3.06 <sup>abc</sup>	0.68 <sup>ab</sup>
SFM Italy	4.49 <sup>abc</sup>	11.75 <sup>de</sup>	1.29 <sup>cde</sup>	3.20 <sup>ab</sup>	0.71 <sup>ab</sup>
Average	4.24	13.60	1.38	2.87	0.68
SEM	0.137	1.288	0.179	0.190	0.040
P-value					
SFM source	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Phytase	0.808	< 0.001	< 0.001	< 0.001	< 0.001
SFE					
Without phytase	4.49	17.12	2.06	2.43	0.54
With phytase	4.42	13.32	1.62	2.80	0.63
P-value	0.927	< 0.001	0.001	0.005	< 0.001
SFM vs. SFE					
SEM	0.630	9.756	0.992	1.040	0.227
P-value	0.024	0.673	0.196	0.882	0.441

<sup>a-e</sup>Means within a row that do not have a common superscript tend to differ ( $P < 0.05$ ).

<sup>1</sup> Data are least squares means of eight observations per treatment. Except for the diets containing the Hungary and U.S. source two, which had seven observations per treatment ( $n = 7$ ). Phytase  $\times$  Sunflower source interactions were not significant; therefore, they were not indicated.

meal (NRC, 2012; Liu et al., 2015; Stein et al., 2016). However, the majority of P in oilseeds is stored as phytic acid, which largely is unavailable for utilization by pigs due to their inability of secreting endogenous phytase (Cowieson et al., 2006; Iyayi et al., 2013).

The observed variation in the concentration of phytate-P in the sunflower co-products used in this experiment (5.8–9.8 g/kg) is likely due to variations in growing or processing conditions; however, one of the SFM sources from the U.S. and one of the SFM sources from Ukraine had a concentration of phytate-P close to the value reported by Rostagno et al. (6.9 g/kg; 2011), but one source of SFM from the U.S. and the SFM from Italy contained less P than previously reported for sunflower co-products (NRC, 2012; Rodríguez et al., 2013; Almeida et al., 2014). The differences in the phytate-P concentration may be due to differences in processing conditions because increased heat during processing of oilseeds may remove some of the P from phytate (Mansour et al., 1993; Agostini et al., 2010).

The ATTD of P in the sunflower co-products determined in this experiment are in agreement with She et al. (2015), but the STTD of P was slightly greater than the STTD of P in sunflower co-products reported previously (NRC, 2012; Rodríguez et al., 2013; Stein et al., 2016), which may be due to differences in the concentration of non-phytate P in the sunflower co-products (Pereira and Adeola, 2016). However, in each of the previous references, only one source of SFM was used and it is, therefore, likely that the present experiment present a more robust dataset for digestibility of P in SFM. The observed lack of differences in ATTD or STTD of P among SFM sources indicates that growing location has minimal impact on the digestibility of P in SFM. This observation is in agreement with data demonstrating that growing location had minimal impact on the digestibility of P in palm kernel meal (Almaguer et al., 2014). Therefore, the hypothesis that growing location of sunflower meal did not influence the STTD of P in sunflower co-products was confirmed, but the observation that ATTD and the STTD of P in SFE without phytase were less than in SFM may indicate that differences regarding processing conditions influence digestibility of P in sunflower co-products. The implication of these observations are that values for the digestibility of P obtained in the U.S. may also be used in Europe and vice versa.

The observed low STTD of P in sunflower co-products is likely due to the high concentration of analyzed phytate-bound P. However, the addition of phytase to diets for pigs partially degrades phytate in the stomach and small intestine, releasing P that can be absorbed (Campbell and Bedford, 1992). The ATTD and STTD of P in SFE and SFM used in this experiment improved substantially with a low inclusion (300 FTU/kg) of phytase in the diets, which is in agreement with reported data (Rodríguez et al., 2013; Almeida et al., 2017; Lee et al., 2021). The practical consequence of this observation is that exogenous phytase improves the efficiency of P in diets for pigs containing SFM or SFE and, at the same time, results in reduced P in manure, which reduces the land area needed for manure application.

All diets contained 9 g per kg of limestone, and assuming that limestone contains 380 g per kg of Ca (NRC, 2012), this amount of limestone contributed approximately 3.49 g per kg of Ca in each diet, which is equivalent to approximately three-quarters of all the Ca in the diets with the remaining one-quarter coming from SFM or SFE. The ATTD of Ca in the diets is, therefore, a combination of the ATTD of Ca in limestone and the ATTD of Ca in SFM or SFE. Concentrations of Ca in the SFM and SFE used in this experiment were

within the range of reported values (NRC, 2012; Rodríguez et al., 2013), but greater than those reported by Zhang et al. (2016) and Rostagno et al. (2011). The ATTD of Ca in diets used in this experiment was in agreement with the values by Rodríguez et al. (2013), but greater than the values reported by Zhang et al. (2016). Because the same quantity of limestone was included in all diets, the small differences among sources of SFM and SFE in the concentration of Ca resulted in small differences among experimental diets. However, these differences were all less than 4 g per kg and therefore, are not believed to have impacted the calculated values for digestibility of Ca and P. Phytase supplementation increased the ATTD of Ca in the diets, which is in agreement with previous data (Rodríguez et al., 2013) and is the result of the liberation of Ca bound to phytate by phytase (Selle et al., 2009).

## 5. Conclusion

Results indicated that adding microbial phytase to pig diets containing sunflower meal and sunflower expellers will increase the digestibility of Ca and P regardless of the type of sunflower co-product used. Sunflower meal had greater apparent total tract digestibility and standardized total tract digestibility of P than sunflower expellers, and small differences in standardized total tract digestibility of P among sunflower meal from different locations were observed, which is likely due to differences in processing conditions of sunflower seeds. Further research with greater levels of phytase inclusion needs to be conducted to determine if greater doses of phytase can improve standardized total tract digestibility of P in sunflower co-products to a greater extent than observed in this experiment.

## Declaration of Competing Interest

The authors have no conflicts of interest.

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