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Effect of phytate, microbial phytase, fiber, and soybean oil on calculated values for apparent and standardized total tract digestibility of calcium and apparent total tract digestibility of phosphorus in fish meal fed to growing pigs[1]

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ABSTRACT: Two experiments were conducted to determine the effects of phytate, phytase, fiber, and soybean oil on apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) of Ca and on ATTD of P in fish meal fed to growing pigs. In Exp. 1, 40 growing pigs (initial average BW: 19.16 ± 2.04 kg) were randomly allotted to 1 of 5 diets with 8 pigs per treatment and placed in metabolism crates. Four diets were used in a 2 × 2 factorial design with 2 levels of phytate (0 or 0.7%) and 2 levels of microbial phytase (0 or 500 phytase units/kg). The diet containing no phytate was based on sucrose, cornstarch, fish meal, casein, and soybean oil, and the diet containing 0.7% phytate was based on corn, corn germ, fish meal, casein, and soybean oil. A Ca-free diet was used to determine basal endogenous losses of Ca. Feces were collected from d 6 to 13 after a 5-d adaptation period. Results indicated that the ATTD and STTD of Ca in fish meal and the ATTD of P increased ($P < 0.001$) if phytase was used and were greater ($P < 0.05$) in the diets based on corn and corn germ. Experiment 2 was conducted to determine the effects of fiber and soybean oil on the ATTD and STTD of Ca and the ATTD of P in fish meal. Fifty growing pigs (initial average BW: 19.36 ± 0.99 kg) were randomly allotted to 1 of 5 diets with 10 pigs per treatment. Two diets contained sucrose, cornstarch, fish meal, casein, and either 0 or 8% of a synthetic source of fiber. Two additional diets contained fish meal, casein, corn, and either 1 or 7% soybean oil. A Ca-free diet was also used. Pigs were housed individually in metabolism crates and fecal samples were collected. Results indicated that fiber increased ($P < 0.001$) the ATTD and STTD of Ca and the ATTD of P, but the ATTD and STTD of Ca or the ATTD of P were not affected by soybean oil. In agreement with the results of Exp. 1, the ATTD and STTD of Ca and the ATTD of P in the corn-based diet were greater ($P < 0.05$) than in the cornstarch-based diet. In conclusion, phytase and fiber increased the ATTD and STTD of Ca and the ATTD of P in fish meal, but inclusion of soybean oil did not affect digestibility of Ca or P. The observation that values for the ATTD and STTD of Ca and ATTD of P are greater in corn-based diets than in cornstarch-based diets indicates that values for the digestibility of Ca and P obtained in cornstarch-based diets may not always be representative for the digestibility in practical corn-based diets.

Key words: calcium digestibility, fiber, fish meal, phytase, phytate, soybean oil

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INTRODUCTION

Phytate may bind Ca from dietary calcium carbonate (González-Vega et al., 2015) and intrinsic Ca in plant ingredients (Selle et al., 2009; González-Vega et al., 2013), but the effect of phytate on the digestibility of Ca in feed ingredients of animal origin has not been reported. Results of experiments in which synthetic forms of phytate were used have been inconsistent (Onyango et al., 2009; González-Vega et al., 2014). However, if natural phytate from an intact feed ingredient is added to a mixed diet that also contains other sources of Ca, phytate-Ca complexes may be formed. When intact feed ingredients are used, not only phytate but also fiber, fat, and other nutrients are often added, and it is not known

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Table 1. Composition of ingredients, as-fed basis

	Ingredient								
	Fish meal		Casein		Corn		Potato protein isolate		Corn
Item[1]	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	germ
GE, kcal/kg	4,274	4,131	5,298	5,337	3,840	3,803	5,241	5,244	6,270
DM, %	92.89	91.47	91.32	92.67	87.73	87.97	91.50	94.43	94.83
CP, %	61.80	62.21	85.37	86.76	7.17	8.62	81.76	81.46	15.03
Ash, %	22.02	22.07	1.69	2.29	1.01	1.43	0.42	3.11	2.42
AEE, %	8.31	8.61	0.91	1.32	2.63	2.88	0.10	0.17	32.88
ADF, %	–	–	–	–	3.66	3.63	–	–	20.23
NDF, %	–	–	–	–	11.40	11.51	–	–	40.15
IDF, %	–	–	–	–	13.00	13.07	–	–	39.56
SDF, %	–	–	–	–	1.66	1.94	–	–	2.79
Ca, %	5.69	5.66	0.03	0.03	ND[2]	0.01	0.04	0.03	0.08
P, %	3.26	3.19	0.59	0.57	0.19	0.23	0.13	0.14	0.58
Phytate, %	–	–	–	–	0.52	0.63	0.33	0.36	1.32
Phytate-bound P[3], %	–	–	–	–	0.15	0.18	0.09	0.10	0.37
Nonphytate P[4], %	3.26	3.19	0.59	0.57	0.04	0.05	0.04	0.04	0.21

[1]AEE = acid-hydrolyzed ether extract; IDF = insoluble dietary fiber; SDF = soluble dietary fiber.

[2]ND = not detected.

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

[4]Nonphytate P was calculated as the difference between total P and phytate-bound P.

to which degree these nutrients influence the standardized total tract digestibility (**STTD**) of Ca and the formation of Ca-phytate bonds.

The digestibility of Ca can be determined by a direct procedure that includes the test ingredient as the only source of the nutrient of interest (Adeola, 2001). By formulating diets based on ingredients that contain virtually no Ca such as cornstarch, corn, or corn germ, it is possible to obtain digestibility values for Ca from the ingredient of interest. However, some fiber and fat is added to the diet if corn or corn germ is used. Fiber may reduce the digestibility of AA and energy (Ma et al., 2008; Zhang et al., 2013), but effects of fiber on the digestibility of minerals is variable (Grieshop et al., 2001). Fat may reduce the absorption of Ca in humans (Agnew and Holdsworth, 1971) and the digestibility of Ca in rats (Frommelt et al., 2014), but to our knowledge, there are no data on the effect of fat on the digestibility of Ca in pigs. The objectives of these experiments were to test the hypothesis that microbial phytase, dietary soybean oil, phytate from corn, and dietary fiber may influence the digestibility of Ca in fish meal.

MATERIALS AND METHODS

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

Experiment 1: Effect of Microbial Phytase on Ca Digestibility in the Absence or in the Presence of Phytate

Diets, animals, and experimental design. Experiment 1 was designed to determine the effect of phytase on the apparent total tract digestibility (**ATTD**) and the STTD of Ca in fish meal in a diet without phytate and in a diet with phytate. Forty growing pigs (initial average BW: 19.16 ± 2.04 kg) were randomly allotted to 1 of 5 diets with 8 replicate pigs per diet and housed individually in metabolism crates. The crates were equipped with a slatted floor, a feeder, a nipple drinker, and a screen floor that allowed for total fecal collection. The Experimental Animal Allotment Program (Kim and Lindemann, 2007) was used to allot pigs to experimental diets.

All dietary ingredients were analyzed for nutrient composition before diet formulation (Table 1). Five diets were formulated. Four diets were used in a 2 × 2 factorial design with 2 levels of phytate (0 and 0.7%) and 2 levels of microbial phytase (0 and 500 phytase units (**FTU**)/kg; Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK). Fish meal was the source of Ca in all diets, and corn and corn germ were used to add natural phytate to the diets (Table 2). A Ca-free diet that contained cornstarch, potato protein isolate, sucrose, soybean oil, Solka Floc, crystalline AA, monosodium phosphate, vitamins, and minerals was also formulated, and this diet was used to determine basal endogenous losses of Ca. All diets except the Ca-free diet were formulated to meet Ca and P requirements of 11- to 25-kg pigs (NRC, 2012; Table 3).

Table 2. Ingredient composition of experimental diets, as-fed basis, Exp. 1

	Diet				
	Fish meal + cornstarch-based diets		Fish meal + corn-corn germ-based diets		
Ingredient, %	0 FTU[1]	500 FTU	0 FTU	500 FTU	Ca-free
Fish meal	12.00	12.00	12.00	12.00	–
Corn	–	–	45.55	45.53	–
Corn germ	–	–	25.00	25.00	–
Cornstarch	50.27	50.25	–	–	47.74
Potato protein isolate	–	–	–	–	20.50
Casein	12.75	12.75	12.75	12.75	–
Sucrose	20.00	20.00	–	–	20.00
Soybean oil	4.00	4.00	4.00	4.00	4.00
Phytase premix[2]	–	0.02	–	0.02	–
Solka Floc[3]	–	–	–	–	5.00
L-Lys HCL	–	–	–	–	0.15
DL-Met	0.16	0.16	–	–	0.14
L-Thr	0.12	0.12	–	–	–
L-His	–	–	–	–	0.12
Monosodium phosphate	–	–	–	–	1.15
Potassium carbonate	–	–	–	–	0.40
Magnesium oxide	–	–	–	–	0.10
Sodium chloride	0.40	0.40	0.40	0.40	0.40
Vitamin mineral premix[4]	0.30	0.30	0.30	0.30	0.30

[1]FTU = phytase units.

[2]The phytase premix contained 2,500 phytase units per gram (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK).

[3]Fiber Sales and Development Corp., Urbana, OH.

[4]The vitamin-micromineral premix provided the following quantities of vitamins and microminerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate; Fe, 126 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn as manganous sulfate, 60.2 mg; Se as sodium selenite and selenium yeast, 0.25 mg; and Zn as zinc sulfate, 124.9 mg.

Table 3. Composition of experimental diets, as-fed basis, Exp. 1

	Diet				
	Fish meal + cornstarch-based diets		Fish meal + corn-corn germ-based diets		
Item	0 FTU	500 FTU[1]	0 FTU	500 FTU	Ca-free
GE, kcal/kg	4,233	4,219	4,861	4,973	4,201
DM, %	92.91	93.13	91.25	91.20	93.19
CP, %	18.24	19.14	25.87	26.97	17.65
Ash, %	3.36	3.33	4.71	4.67	2.05
AEE[2], %	1.76	1.43	15.68	14.28	1.82
ADF, %	0.39	0.34	4.04	5.96	5.83
NDF, %	3.11	2.61	19.35	21.08	5.35
Ca, %	0.77	0.73	0.64	0.70	0.02
P, %	0.51	0.49	0.66	0.70	0.36
Phytase, FTU/kg	ND[3]	667	ND	712	<50
Calculated values					
ME, kcal/kg	3,940	3,939	3,659	3,659	3,761
Phytate[4], %	ND	ND	0.57	0.57	0.07
Phytate-bound P[5], %	–	–	0.16	0.16	0.02
Nonphytate P[6], %	0.51	0.49	0.50	0.54	0.34
SID[7]	1.32	1.32	1.54	1.54	1.24

[1]FTU = phytase units.

[2]AEE = acid-hydrolyzed ether extract.

[3]ND = not detected.

[4]Phytate values were calculated using the values of phytate in the ingredients.

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

[6]Nonphytate P was calculated as the difference between total P and phytate-bound P.

[7]SID=standardized ileal digestible.

Feeding and sample collection. Each pig was fed one of the experimental diets for 13 d, and an amount of diet equivalent to 2.7 times the daily maintenance energy requirement (i.e., 197 kcal of ME/kg BW$^{0.60}$; NRC, 2012) was provided each day. Pigs had free access to water throughout the experiment. The daily allotments of feed were divided into 2 equal meals and provided at 0700 and 1700 h. Pigs had a 5-d adaptation period to the diets followed by 5 d of quantitative collection of fecal samples according to the marker-to-marker approach (Adeola, 2001). An indigestible marker (Indigo carmine) was added to the morning meal on d 6 to mark the beginning of fecal collection, and ferric oxide was added to the morning meal on d 11 to mark the conclusion of fecal collection. The ferric oxide marker passed on d 12 or 13, and fecal collections ceased at the time this marker appeared in the feces. Fecal samples were stored at −20°C immediately after collection. Orts that were collected during the collection period were dried in a forced-air oven at 65°C, and the weight was subtracted from the total feed intake.

Sample Analysis

Fecal samples were dried in a forced-air oven at 65°C, ground in a Wiley mill (Model 4; Thomas Scientific, Swedesboro, NJ) using a 1-mm screen, and subsamples were collected for analysis after all the ground materials had been mixed. Fish meal, casein, corn germ, corn, potato protein isolate, diets, and fecal samples were analyzed for DM by oven drying at 135°C for 2 h (Method 930.15; AOAC, 2007) and for Ca and P by inductively coupled plasma-optical emission spectroscopy (**ICP–OES**; Method 985.01A, B, and D; AOAC, 2007) after wet ash sample preparation (Method 975.03 B[b]; AOAC, 2007). Fish meal, casein, corn germ, corn, potato protein isolate, and diet samples were analyzed for GE using an isoperibol bomb calorimeter (Model 6300; Parr Instruments, Moline, IL) and for N using the combustion procedure (Method 990.03; AOAC, 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ), and CP was calculated as N × 6.25. These samples were also analyzed for ash (Method 942.05; AOAC, 2007) and for acid-hydrolyzed ether extract using 3*N* HCl (Sanderson, 1986) followed by crude fat extraction with petroleum ether (Method 2003.06; AOAC, 2007) on a Soxtec 2050 automated analyzer (FOSS North America, Eden Prairie, MN). Corn germ and corn were analyzed for ADF and NDF using Ankom Technology method 12 and 13, respectively (Ankom 2000 fiber analyzer; Ankom Technology, Macedon, NY). Diets were analyzed for ADF (Method 973.18; AOAC, 2007) and NDF (Holst, 1973). Corn germ, corn, and potato protein isolate were also analyzed for phytate (Ellis et al., 1977). Diets were analyzed for phytase activity (Engelen et al., 2001). Corn and corn germ were analyzed for insoluble dietary fiber (**IDF**) and soluble dietary fiber (**SDF**) using the Ankom dietary fiber analyzer (Method 991.43; AOAC, 2007). Corn germ was defatted before analyzed for IDF and SDF (Method 985.29E; AOAC, 2007).

Calculations and Statistical Analysis

The concentration of phytate-bound P in corn germ, corn, potato protein isolate, and diets was calculated as 28.2% of phytate (Tran and Sauvant, 2004) and the concentration of nonphytate P was calculated by subtracting phytate-bound P from total P.

The basal endogenous losses of Ca were determined from pigs fed the Ca-free diet, and ATTD and STTD values of Ca in each diet were calculated as outlined for calculation of ATTD and STTD of P (Almeida and Stein, 2010; NRC, 2012).

The UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC) was used to determine normality of residuals and also to identify outliers. One pig fed the fish meal-cornstarch-based diet with phytase was identified as an outlier and removed from the data set. Data were analyzed as a 2 × 2 factorial using Proc MIXED of SAS. The model included the fixed effects of type of diet, phytase, and the interaction between type of diet and phytase. However, only 3 interactions were observed, and the final model, therefore, contained only the fixed effects of diet and phytase. The LSMEANS procedure was used to calculate the mean values for the treatments, and means were separated using the PDIFF option if significant differences were observed. Pig was the experimental unit and an α level of 0.05 was used to assess significance among treatments.

Experiment 2: Effects of Type of Diet, Fiber, and Soybean Oil on Ca Digestibility

Diets, animals, and experimental design. Experiment 2 was designed to evaluate the effects of fiber and soybean oil on digestibility of Ca in fish meal. Fifty growing pigs (initial average BW: 19.36 ± 0.99 kg) were randomly allotted to 1 of 5 diets. There were 2 blocks of 25 pigs with 5 replicate pigs for each diet in each block. Therefore, a total of 10 pigs were used per treatment. Pigs were housed individually in metabolism crates as described for Exp. 1.

A cornstarch-based diet containing fish meal as the sole source of Ca was formulated, and a similar diet was formulated with the exception that 8% Solka Floc was included at the expense of cornstarch to evaluate

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

	Diet				
Ingredient, %	Fish meal-cornstarch	Fish meal-cornstarch + fiber	Fish meal-corn	Fish meal-corn + soybean oil	Ca-free
Fish meal	12.00	12.00	12.00	12.00	–
Casein	12.75	12.75	12.75	12.75	–
Potato protein isolate	–	–	–	–	17.00
Cornstarch	53.37	45.37	–	–	–
Corn	–	–	73.65	67.65	80.24
Sucrose	20.00	20.00	–	–	–
Soybean oil	1.00	1.00	1.00	7.00	1.00
Solka Floc[1]	–	8.00	–	–	–
Monosodium phosphate	–	–	–	–	0.97
L-Lys HCL	–	–	–	–	0.16
DL-Met	0.16	0.16	–	–	0.01
L-Thr	0.12	0.12	–	–	–
L-His	–	–	–	–	0.02
Sodium chloride	0.40	0.40	0.40	0.40	0.40
Vitamin mineral premix[2]	0.20	0.20	0.20	0.20	0.20

[1]Fiber Sales and Development Corp., Urbana, OH.

[2]The vitamin-micromineral premix provided the following quantities of vitamins and microminerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu as copper sulfate, 20 mg; Fe as iron sulfate, 126 mg; I as ethylenediamine dihydriodide, 1.26 mg; Mn as manganous sulfate, 60.2 mg; Se as sodium selenite and selenium yeast, 0.25 mg; and Zn as zinc sulfate, 124.9 mg.

the effects of fiber on ATTD and STTD of Ca (Table 4). Two corn-based diets in which fish meal was the sole source of Ca were also formulated with 2 levels of soybean oil (1 or 7%) to evaluate the effect of soybean oil on ATTD and STTD of Ca. All diets were formulated to meet Ca and P requirements for 11- to 25-kg pigs (NRC, 2012; Table 5). A Ca-free diet that contained corn, potato protein isolate, soybean oil, monosodium phosphate, crystalline AA, vitamins, and minerals was also formulated to determine basal endogenous losses of Ca. Each pig was fed one of the experimental diets for 13 d with a 5-d adaptation period and a 5-d marker-based fecal collection period as explained for Exp. 1. An amount of diet equivalent to 3 times the daily maintenance energy requirement was provided each day.

Fish meal, casein, corn, potato protein isolate, diets, and fecal samples were analyzed for DM, Ca, and P. Diets and ingredients were analyzed for GE, CP, acid-hydrolyzed ether extract, and ash. Corn and diets were analyzed for ADF, NDF, and phytate. Corn was analyzed for SDF and IDF as explained for Exp. 1. Values for ATTD and STTD of Ca in each diet were calculated as explained for Exp. 1.

Statistical Analysis

The UNIVARIATE procedure of SAS (SAS Inst. Inc.) was used to determine the normality of residuals and also to identify outliers. Three outliers were identified and removed from the data (2 pigs fed the fish meal diet and 1 pig fed the fish meal with fiber diet). Data were analyzed using the Proc MIXED of SAS with diet as the fixed effect and period as the random effect, and CONTRAST statements were used to determine differences between cornstarch and corn-based diets, the effect of fiber, and the effect of soybean oil on response variables. The LSMEANS procedure was used to calculate means as explained for Exp. 1.

RESULTS

Experiment 1: Effect of Microbial Phytase on Ca Digestibility in the Absence or the Presence of Phytate

All pigs remained healthy and consumed their diets during the experiment. Feed intake was greater ($P < 0.01$) for pigs fed corn-corn germ-based diets than for pigs fed cornstarch-based diets, but ADFI was not affected by the inclusion of phytase (Table 6). Daily Ca intake was not affected by the type of diet used or by inclusion of phytase in the diets. Daily fecal output increased ($P < 0.001$) if corn-corn germ-based diets were used, but decreased ($P < 0.05$) if phytase was added to the diets. The concentration of Ca in the feces and the output of Ca decreased ($P < 0.001$) if corn-corn germ-based diets rather than cornstarch-based diets were used, and the output of Ca in feces was less ($P < 0.05$) if phytase was added to the diets than if no phytase was used. The amount of Ca absorbed, ATTD of Ca, and STTD of Ca increased ($P <$

Table 5. Composition of experimental diets, as-fed basis, Exp. 2

	Diet				
Item	Fish meal-cornstarch	Fish meal-cornstarch + fiber	Fish meal-corn	Fish meal-corn + soybean oil	Ca-free
DM, %	93.54	93.88	88.77	89.89	89.27
Ash, %	3.44	3.28	4.14	4.06	2.64
GE, kcal/kg	4,044	4,034	4,027	4,407	4,277
CP, %	19.01	18.70	23.68	24.28	22.19
NDF, %	1.15	6.32	7.08	8.10	7.47
ADF, %	0.09	3.74	2.40	2.39	3.34
AEE[1], %	2.30	2.06	4.20	9.22	3.74
Ca, %	0.71	0.72	0.74	0.68	0.03
P, %	0.48	0.49	0.64	0.60	0.48
Calculated values					
ME, kcal/kg	3,811	3,492	3,476	3,775	3,488
Phytate[2], %	ND[3]	ND	0.46	0.43	0.57
Phytate-bound P[4], %	–	–	0.13	0.12	0.16
Nonphytate P[5], %	0.48	0.49	0.51	0.48	0.32
SID Lys[6], %	1.32	1.32	1.47	1.46	1.22

[1]AEE = acid-hydrolyzed ether extract.

[2]Phytate values were calculated using the values of phytate in the ingredients.

[3]ND = not detected.

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

[5]Nonphytate P was calculated as the difference between total P and phytate-bound P.

[6]SID=standardized ileal digestible.

0.001) if corn-corn germ-based diets were used compared with cornstarch-based diets and were greater ($P < 0.05$) if phytase was added to the diets than if no phytase was used. Daily basal endogenous losses of Ca increased ($P < 0.05$) in cornstarch-based diets if phytase was used but decreased ($P < 0.05$) in corn-corn germ-based diets if phytase was included (diet × phytase interaction; $P < 0.05$).

Phosphorus intake was greater ($P < 0.001$) if corn-corn germ-based diets were used than that if pigs were fed cornstarch-based diets. However, the concentration of P in feces was less ($P < 0.05$) for pigs fed corn-corn germ-based diets than that for pigs fed cornstarch-based diets and was not affected by the inclusion of phytase. Daily P output decreased ($P < 0.01$) if phytase was added to the diets to a greater extent if corn-corn germ-based

Table 6. Apparent total tract digestibility (ATTD) of Ca and P and standardized total tract digestibility (STTD) of Ca in diets containing fish meal-cornstarch or fish meal-corn-corn germ without or with microbial phytase, Exp. 1

	Diet		Phytase			*P*-value	
Item	Fish meal-cornstarch	Fish meal-corn-corn germ	0 FTU[1]	500 FTU	SEM	Diet	Phytase
ADFI, g/d	762	824	824	825	14	0.004	0.361
Ca intake, g/d	5.71	5.52	5.50	5.73	0.10	0.186	0.094
Fecal output, g/d	25.67	148.13	91.11	82.69	2.86	<0.001	0.049
Ca in feces, %	10.74	0.85	6.09	5.50	0.29	<0.001	0.161
Fecal Ca output, g/d	2.60	1.17	2.09	1.68	0.11	<0.001	0.016
Ca absorbed, g/d	3.11	4.35	3.41	4.05	0.15	<0.001	0.004
ATTD of Ca, %	54.24	78.54	62.15	70.64	2.12	<0.001	0.009
Basal ECaL[2,3], mg/d	156	168	161	163	2.06	<0.001	0.697
STTD of Ca, %	56.97	81.54	65.04	73.48	2.12	<0.001	0.009
P intake, g/d	3.81	5.70	4.71	4.80	0.06	<0.001	0.311
P in feces, %	5.47	0.88	3.35	3.00	0.13	<0.001	0.063
Fecal P output[3], g/d	1.30	1.23	1.43	1.11	0.07	0.484	0.003
P absorbed[3], g/d	2.50	4.37	3.19	3.69	0.09	<0.001	<0.001
ATTD of P, %	65.62	77.88	68.21	75.29	1.57	<0.001	0.004

[1]FTU = phytase units.

[2]ECaL = endogenous losses of Ca. Values for the STTD of Ca were calculated by correcting ATTD values for basal endogenous losses. Basal endogenous losses were determined from pigs fed the Ca-free diet as 0.22 g/kg DMI.

[3]Diet by phytase interaction was significant ($P < 0.05$).

Table 7. Apparent total tract digestibility (ATTD) of Ca and P and standardized total tract digestibility (STTD) of Ca, Exp. 2

	Diet					Contrast *P*-value		
Item	Fish meal-corn-starch (1)	Fish meal-corn-starch-fiber (2)	Fish meal-corn (3)	Fish meal-corn-soybean oil (4)	SEM	Cornstarch vs. corn (1 vs. 3)	Fiber (1 vs. 2)	Soybean oil (3 vs. 4)
ADFI, g/d	867	907	982	905	14.57	<0.001	0.049	<0.001
Ca intake, g/d	6.16	6.53	7.26	6.15	0.10	<0.001	0.011	<0.001
Fecal output, g/d	28.97	72.63	87.43	72.46	2.29	<0.001	<0.001	<0.001
Ca in feces, %	12.48	3.87	1.32	1.45	0.16	<0.001	<0.001	0.443
Fecal Ca output, g/d	3.66	2.82	1.15	1.05	0.19	<0.001	<0.001	0.616
Ca absorbed, g/d	2.48	3.71	6.12	5.10	0.14	<0.001	<0.001	<0.001
ATTD of Ca, %	40.42	57.08	84.24	82.91	2.51	<0.001	<0.001	0.623
Basal ECaL[1], mg/d	321	337	345	322	5.30	0.001	0.031	<0.001
STTD of Ca, %	45.64	62.23	88.99	88.14	2.51	<0.001	<0.001	0.754
P intake, g/d	4.16	4.44	6.28	5.43	0.08	<0.001	0.011	<0.001
P in feces, %	6.52	2.14	1.71	1.74	0.12	<0.001	<0.001	0.765
Fecal P output, g/d	1.88	1.55	1.49	1.27	0.10	0.001	0.009	0.043
P absorbed, g/d	2.27	2.89	4.79	4.16	0.08	<0.001	<0.001	<0.001
ATTD of P, %	54.66	65.18	76.29	76.70	1.84	<0.001	<0.001	0.845

[1]ECaL = endogenous losses of Ca. Values for the STTD of Ca were calculated by correcting ATTD values for basal endogenous losses. Basal endogenous losses were determined from pigs fed the Ca-free diet as 0.396 g/kg DMI.

diets were used than if cornstarch-based diets were used (diet × phytase interaction; $P < 0.05$). Therefore, inclusion of phytase increased ($P < 0.01$) the amount of P absorbed in corn-corn germ-based diets but not in cornstarch-based diets (diet × phytase interaction; $P < 0.05$). The ATTD of P was greater ($P < 0.001$) in corn-corn germ-based diets than that in cornstarch-based diets and was also greater ($P < 0.05$) if phytase was included in the diets than that if no phytase was used.

Experiment 2: Effects of Type of Diet, Fiber, and Soybean Oil on Ca Digestibility

Pigs readily consumed their diets throughout the experiment, but 1 pig fed the fish meal with fiber diet died on d 4 of the adaptation period due to meningitis of bacterial origin. All other pigs remained healthy throughout the experiment.

Type of diet: cornstarch-based diet vs. corn-based diet. Feed intake, Ca intake, fecal output, Ca absorbed, basal endogenous losses of Ca, the ATTD of Ca, and the STTD of Ca were greater ($P < 0.05$) for pigs fed the corn-based diets than for pigs fed the cornstarch-based diets (Table 7). However, Ca in feces and the amount of Ca output was greater ($P < 0.05$) from pigs fed the cornstarch-based diets than from pigs fed the corn-based diets. Pigs fed the corn-based diets had greater ($P < 0.05$) P intake, P absorbed, and greater ATTD of P than those of pigs fed cornstarch-based diets, but P-output in feces was less ($P < 0.05$) for pigs fed corn-based diets compared with pigs fed cornstarch-based diets.

Effect of fiber. Feed intake, daily Ca intake, fecal output, Ca absorbed, basal endogenous losses of Ca, ATTD of Ca, and STTD of Ca increased ($P < 0.001$) if fiber was included in the cornstarch-based diet. Fiber also reduced ($P < 0.001$) the amount and percentage of Ca in feces. Inclusion of fiber increased ($P < 0.001$) P intake, P absorbed, and the ATTD of P but decreased ($P < 0.001$) the amount and percentage of P in feces.

Effect of soybean oil. Inclusion of soybean oil in the corn-based diet reduced ($P < 0.001$) feed intake, Ca intake, fecal output, the amount of Ca absorbed, and basal endogenous losses of Ca but did not affect the amount and percentage of Ca in feces or the ATTD or STTD of Ca. Phosphorus intake, fecal P output, and the amount of P absorbed decreased ($P < 0.001$) if soybean oil was included in the diet, but the percentage of P in feces and the ATTD of P were not affected by inclusion of soybean oil in the diet.

DISCUSSION

Chemical Characteristics of Ingredients

The concentrations of Ca and P in the fish meal used in these 2 experiments were greater than reported values (Rostagno et al., 2011; NRC, 2012; Rojas and Stein, 2013), but were in agreement with values reported by Sauvant et al. (2004). Increased Ca and P concentrations in fish meal were likely the result of inclusion of more bone from the fish filet industry in fish meal, which increases the concentration of ash in fish meal. The concentration of Ca in casein and the concentrations of Ca, P, and phytate in potato protein isolate concurred with reported values (Cervantes-Pahm and Stein, 2010; Rostagno et al., 2011; NRC, 2012; González-Vega et al., 2013). Because of the negligible Ca in casein and potato protein isolate, these ingredients can be used as AA sources in diets that are formulated to determine Ca digestibility. The concentrations of Ca, P, and phytate in corn were within the range of published values (Rostagno et al., 2011; NRC, 2012; Rojas et al., 2013) and confirm that corn and corn-coproducts contain virtually no Ca and that most P is bound to phytate.

Endogenous Losses of Ca

Basal endogenous loss of Ca obtained from pigs fed the Ca-free diet based on cornstarch, potato protein isolate, and sucrose was 0.22 g/kg DMI (Exp. 1), but the endogenous loss from pigs fed the Ca-free diet based on corn and potato protein isolate was 0.396 g/kg DMI (Exp. 2). The reason for this difference may be that different ingredients used in a nutrient-free diet may result in different endogenous losses of nutrients (Boisen and Moughan, 1996).

Experiment 1: Effect of Microbial Phytase on Ca Digestibility in the Absence or the Presence of Phytate

The reason for the greater feed intake in pigs fed corn-corn germ-based diets compared with that of pigs fed cornstarch-based diets is that these diets contained less ME than that of the cornstarch-based diets and because pigs were fed to the same daily ME intake, more of the corn-corn germ-based diets was provided. The observation that inclusion of microbial phytase increased Ca and P digestibility in corn-corn germ-based diets was expected because phytase hydrolyzes phytate, which increases the availability of P and Ca to be absorbed (Poulsen et al., 2010; Almeida et al., 2013; Rodríguez et al., 2013; Almaguer et al., 2014). Because all Ca in the diets was from fish meal, the increased ATTD of Ca that was observed as phytase was added to the diets indicates that Ca from fish meal was bound to phytate, which to our knowledge has not been previously reported. It is, however, not clear why the ATTD and STTD of Ca in the cornstarch diet were increased by phytase because this diet did not contain phytate. The values for ATTD of Ca in fish meal that were calculated were close to the reported values for ATTD of Ca in diets containing fish bones fed to pigs (Malde et al., 2010). However, to our knowledge, this is the first time values for the STTD of Ca in fish meal have been reported.

Phytate reduces the digestibility not only of P but also of Ca (Selle et al., 2009). Pigs fed corn-soybean meal diets had less ATTD of Ca and P than that of pigs fed a corn grits diets because of the greater concentration of phytate in the corn-soybean meal diets than in the corn grits diets (Liu et al., 2014). It was, therefore, expected that the ATTD of Ca and P in the corn-corn germ-based diets would be less than that in the cornstarch-based diets. However, in contrast with our hypothesis, an increase in ATTD and STTD of Ca and in the ATTD of P in the corn-corn germ-based diet was observed compared with those of the cornstarch-based diet. It may be speculated that the reason for this observation is that the fiber in the corn-corn germ-based diet increased the motility in the intestinal tract and, thereby, enhanced absorption and reduced precipitation of minerals, but further research is needed to verify this hypothesis.

It is also possible that the digestibility of Ca and P was increased because of the greater concentration of fat in the corn-corn germ-based diets than in the cornstarch-based diets because dietary fat may decrease the rate of passage, which may enhance the digestibility of nutrients (Cervantes-Pahm and Stein, 2008; Kil and Stein, 2011). The Ca:STTD P ratio was similar among all diets, but the Ca:total P ratio was 1.5:1 for the cornstarch-based diets and 1:1 for the corn-corn germ-based diets. Decreasing the Ca:total P ratio from 1.5:1 to 1:1 may increase the ATTD of Ca and P in corn-based diets containing microbial phytase (Liu et al., 1998).

Values for the ATTD of P in the corn-corn germ-based diet were a combination of the ATTD of P in fish meal and the ATTD of P in corn and corn germ. Reported values for the ATTD of P in fish meal range from 62.7 to 90.0% (Rodehutscord et al., 1997; NRC, 2012; Sulabo et al., 2013; Kim et al., 2014). In corn, the range in ATTD of P is from 26.0 to 33.5% (Almeida and Stein, 2012; NRC, 2012; Rojas et al., 2013), and in corn germ, values for ATTD of P range from 33.0 to 37.3% (Almeida and Stein, 2012; NRC, 2012). Therefore, corn-corn germ-based diets were expected to have less ATTD of P than the cornstarch-based diets because most of the P in the cornstarch-based diets originated from fish meal. It is possible that the reason we were not able to verify this hypothesis is that the lack of fiber in the cornstarch-based diets may have resulted in precipitation of the P from fish meal, which resulted in reduced digestibility in this diet.

Experiment 2: Effects of Type of Diet, Fiber, and Soybean Oil on Ca Digestibility

Experiment 2 was conducted to elucidate possible reasons for the unexpected results from Exp. 1, which indicated that the ATTD and STTD of Ca and the ATTD of P are greater in corn-corn germ-based diets than in cornstarch-based diets. Results of Exp. 2 confirmed that values for ATTD and STTD of Ca and the ATTD of P were greater in the corn-based diets than in the cornstarch-based diets. The values for ATTD and STTD of Ca (40.42 and 45.64%, respectively) in fish meal in the cornstarch-based diet obtained in this experiment were close to the values for ATTD and STTD of Ca (54.24 and 56.97%, respectively) in fish meal observed in Exp. 1. In contrast, the ATTD and STTD of Ca in the corn-based diet was 84.24 and 88.99%, respectively.

Because all Ca in all diets was from fish meal, the difference in ATTD and STTD of Ca between the 2 types of diets may have been caused by fiber, fat, or other unknown factors. The fact that fiber (Solka Floc) and corn increased the ATTD and STTD of Ca in fish meal by 16.6 and 42.5% units, respectively, indicates that fiber possibly prevented some precipitation that may have occurred in the cornstarch-based diet. However, Solka Floc is 100% cellulose, which is insoluble fiber, whereas the total dietary fiber in corn is 28% soluble and 72% insoluble (Jaworski et al., 2015). Fermentation of soluble dietary fiber results in production of short chain fatty acids, which may contribute to increased mineral absorption due to a reduction in intestinal pH (Wong et al., 2006; Rose et al., 2007). A low pH may enhance Ca solubility and increase absorption of Ca in rats (Ohta et al., 1995), humans (Coudray et al., 1997), and pigs (Bird et al., 2000). Synthesis of butyrate may also enhance the growth of epithelial cells in the small and large intestine, which may further enhance absorption of nutrients (Montagne et al., 2003). Therefore, the greater digestibility of Ca observed in the corn-based diet compared with that of the cornstarch-based diet may be a result of fermentation of fiber in the corn-based diet.

The high values for STTD of Ca in the corn-based diet indicate that Ca from fish meal has a high digestibility in commercial diets. The calculated value for ATTD of Ca in fish meal in the cornstarch-fiber diet was in agreement with reported values, but the value in the corn-based diet was greater than of previously reported values of diets containing fish bones (Malde et al., 2010).

A positive effect of fat on digestibility of AA in pigs has been observed, which is presumed to be a result of a reduction in the rate of passage (Cervantes-Pahm and Stein, 2008; Kil and Stein, 2011). However, the effect of fat on the ATTD of Ca and P in rats and humans is variable because depending on the type of fatty acids, different effects on pH and formation of Ca soaps have been reported (Boyd et al., 1932; Agnew and Holdsworth, 1971; Wargovich et al., 1984). In humans, high levels of Ca increased fat excretion (Bendsen et al., 2008), which may lead to a lower body fat content (Soares et al., 2012). In rats, high dietary fat content decreases the digestibility of Ca due to formation of Ca soaps (Frommelt et al., 2014). Saturated fat decreases the absorption of Ca (Gacs and Barltrop, 1977). However, soybean oil is mostly unsaturated fat, which may result in less Ca soaps being formed. This may be the reason for the observation in the present experiment indicating that soybean oil did not influence the ATTD and STTD of Ca or the ATTD of P in pigs.

Conclusions

The digestibility of Ca and P in fish meal is less in cornstarch-based diets than in corn-based diets, but inclusion of microbial phytase in the diet will increase the digestibility of Ca and P regardless of the type of diet used. Inclusion of synthetic fiber in the cornstarch-based diet resulted in an increased digestibility of Ca and P, and it is possible that the lack of fiber in cornstarch-based diets results in precipitation of Ca and P in the intestinal tract. Soybean oil did not affect the ATTD and STTD of Ca or the ATTD of P in fish meal. Due to the negative effect of cornstarch-based diets on the ATTD and STTD of Ca, it is recommended that Ca digestibility of feed ingredients should be determined in corn-based diets.

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