Journal of Animal Science, 2020, Vol. 98, No. 3, 1–12

doi:10.1093/jas/skaa053
Advance Access publication February 15, 2020
Received: 3 January 2020 and **Accepted:** 12 February 2020
Non Ruminant Nutrition

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Phytate degradation cascade in pigs as affected by phytase supplementation and rapeseed cake inclusion in corn–soybean meal-based diets

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Abstract

Two experiments (**Exp.**) with ileally cannulated growing barrows were conducted. The concentrations of positional inositol phosphate (**InsP**) isomers in ileal digesta and feces were determined, as well as the prececal and total tract phytate ($\mathbf{InsP_6}$) hydrolysis, and digestibility of dry matter, P, Ca, nitrogen, and gross energy. Prececal amino acid (**AA**) digestibility and digestive enzyme activities in ileal digesta were also studied. In both Exp., pigs had an initial body weight (**BW**) of 28 kg and were completely randomized to a Double Latin Square Design with eight pigs, four diets, and three periods of 12 d each. Feces and ileal digesta were collected for 5 d and 2 d, respectively. Pigs were housed individually in stainless steel metabolic units. Water was available ad libitum and feed was provided two times daily at an amount of 4% of mean BW. In Exp. 1, pigs received a corn–soybean meal (**SBM**)-based diet that was supplemented with 0, 750, 1,500, or 3,000 FTU of a microbial phytase/kg diet. In Exp. 2, pigs were allotted to a 2 × 2 arrangement of diets based on corn and SBM or an SBM-rapeseed cake (**RSC**) mix and phytase supplementation at 0 or 1,500 FTU/kg of diet. In ileal digesta of pigs fed without the phytase supplement, the dominating InsP isomers beside $InsP_6$ were $InsP_5$ isomers. The InsP pattern in ileal digesta changed with the inclusion of microbial phytase in both Exp., as there was a remarkable increase in Ins(1,2,5,6)P_4 concentration ($P < 0.001$). In both Exp., the *myo*-inositol concentration in ileal digesta was greater upon phytase addition ($P < 0.001$). Without phytase supplementation, prececal and total tract P digestibility were low, whereas hardly any $InsP_6$ was excreted in feces. There was no difference between prececal and total tract P digestibility values. For most AA studied in Exp. 2, prececal digestibility was lower ($P < 0.01$) when the diet contained RSC. However, phytase supplementation did not significantly affect prececal AA digestibility in both Exp. The present study showed that $InsP_6$ disappearance by the end of the ileum can be increased up to around 90% in SBM- and SBM–RSC-based diets when microbial phytase is supplemented, but prececal P digestibility hardly exceeded 60%. The study confirms that pigs cannot benefit from a remarkable $InsP_6$ degradation in the hindgut.

Key words: digestive enzymes, growing pigs, inositol phosphates, *myo*-inositol, phytate hydrolysis, protein source

Abbreviations

AA	amino acid
ADF	acid detergent fiber
ADL	acid detergent lignin
BD	basal diet
BD_{SBM}	basal diet based on corn and soybean meal
BD_{RSC}	basal diet based on corn, soybean meal and rapeseed cake
BW	body weight
CF	crude fiber
CP	crude protein
DM	dry matter
EE	ether extract
Exp	experiment
FTU	phytase units
GE	gross energy
InsP	inositol phosphates
$InsP_6$	*myo*-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate)
N	nitrogen
NDF	neutral detergent fiber
RSC	rapeseed cake
RSM	rapeseed meal
SBM	soybean meal

Introduction

In plant seeds, phytic acid (*myo*-inositol 1,2,3,4,5,6-hexakis [dihydrogen phosphate] [**$InsP_6$**]) and its salts are the major storage form of P. Pigs are known to be nearly unable to hydrolyze $InsP_6$ in the digestive process due to a lack of significant endogenous phytase activity and low microbial population in the upper part of the gastrointestinal tract (Schlemmer et al., 2001). Phytases are widely used as additives in pig nutrition to avoid or reduce this limitation (Selle and Ravindran, 2008). However, a recent meta-analysis concluded that the average P digestibility in pigs plateaued at 65% with increasing supplementation of phytases (Rosenfelder-Kuon et al., 2020), although $InsP_6$ recovery in feces of pigs is very low (Baumgärtel et al., 2008; Rutherfurd et al., 2014). There is a scarcity of information on the stepwise degradation pathway of $InsP_6$ to lower inositol phosphate (**InsP**) isomers in the gastrointestinal tract of pigs (Rodehutscord and Rosenfelder, 2016). Furthermore, $InsP_6$ hydrolysis was mostly determined in diets with soybean meal (**SBM**) as a protein source (Kühn et al., 2016; Laird et al., 2016, 2018). However, rapeseed cake (**RSC**) and other rape byproducts are also used as protein feedstuffs for pigs (Kaewtapee et al., 2018), but they contain more $InsP_6$ than SBM. Until now, no studies were conducted to compare SBM and RSC as protein sources considering $InsP_6$ hydrolysis and the concentration of lower InsP in the gastrointestinal tract of pigs. Anti-nutritional effects of $InsP_6$, such as a decreased gross energy (**GE**), nitrogen (**N**), and amino acid (**AA**) digestibility, and a reduced utilization of other macro- and micro-nutrients, are also discussed in the literature (Selle and Ravindran, 2008). Hence, $InsP_6$ degradation in the gastrointestinal tract should have the potential to reduce such anti-nutritional effects.

The first objective of the present study was to investigate $InsP_6$ degradation and concentrations of lower InsP isomers and *myo*-inositol in ileal digesta and feces of pigs fed graded levels of phytase. Secondly, the differences when using SBM alone or a mix of SBM and RSC containing more $InsP_6$ as protein sources were investigated. The prececal digestibility of AA and prececal and total tract digestibility of dry matter (**DM**), N, GE, P, and Ca was also studied. Finally, the activity of different proteolytic enzymes in ileal digesta was analyzed when the different protein feeds were used.

Materials and Methods

The research protocol was approved by the Regierungspräsidium Stuttgart, Germany, in accordance with the German Animal Welfare Legislation (approval No. V 339/17 TE). Care of the animals throughout this experiment (**Exp.**) was in accordance with the corresponding Directive 2010/63/EU (European Parliament and the Council of the European Union, 2010).

Animals, housing, experimental diets, and design

Two Exp. were conducted. In Exp. 1 and 2, eight growing barrows (German Landrace × Piétrain) each were obtained from the University Agriculture Research Station (Unterer Lindenhof, Eningen unter Achalm, Germany). The pigs were housed individually in stainless steel metabolic units (0.8 by 1.5 m) in an experimental room that was equipped with an automated temperature control system and adjusted to a room temperature of 20 °C. Each metabolic unit was equipped with two infrared heating lamps. A low-pressure drinking nipple allowed free access to drinking water. For each Exp., on days 4 and 6 after arrival in the experimental room, four pigs each day were surgically fitted with a T-cannula at the distal ileum as described by Li et al. (1993). After surgery, pigs were allowed a recovery period of at least 7 d. The age of pigs at the beginning of the Exp. was 12 wk and pigs' initial body weight (**BW**) was 27.6 ± 1.8 and 27.8 ± 1.7 kg for Exp. 1 and 2, respectively. The BW at the end was 46.6 ± 2.6 and 47.1 ± 4.2 kg for Exp. 1 and 2, respectively.

Daily feed allowance was 4% of mean BW of all pigs. This corresponds to three times the estimated energy requirement for maintenance (i.e., 824 kJ ME/$kg^{0.60}$ BW [NRC, 2012]). Pigs were weighed at the beginning of each period and the amount of feed was adjusted accordingly. Daily feed allotments were divided into two equal parts and provided at 0715 and 1915 hours. Diets were used in mash form and mixed with hand warm water at a ratio of 1:1 (w/w) immediately before delivery to the pig.

Feed ingredients used for mixing the diets (corn, SBM, and RSC) were ground through a 2-mm sieve before mixing. The phytase used in both Exp. was an *E.coli*-derived 6-phytase (Quantum Blue, AB Vista, UK). All diets contained on as-fed basis 5 g/kg of titanium dioxide as an indigestible marker, 0.3 g/kg vitamin E as an antioxidant, and 20 g/kg of a P-free mineral and vitamin premix (BASU Mineralfutter GmbH, Germany). The ingredient composition of the basal diets (**BDs**) is presented in Table 1. In Exp. 1, a corn–SBM-based BD was formulated to meet the requirements for growing pigs at 30 to 50 kg BW according to the recommendations of the Gesellschaft für Ernährungsphysiologie (2006) with the exception of P. Corn and SBM were chosen because they have a low content of P and low intrinsic phytase activity. The BD was divided and three additional diets were formulated to contain 750, 1,500, and 3,000 FTU/kg. In Exp. 2, two BDs based on either corn and SBM (**BD_{SBM}**) or corn, SBM, and RSC (**BD_{RSC}**) were formulated to meet the requirements for 30 to 50 kg BW according to the recommendations of the Gesellschaft für Ernährungsphysiologie (2006) with the exception of P. To meet the crude protein (**CP**) and AA requirements and to avoid negative effects on feed intake due to excessive RSC inclusion, SBM was only partially substituted by RSC (20% inclusion rate). Diets were fed without or with the inclusion of 1,500 FTU/kg phytase. This level of phytase was chosen based on the results of Exp. 1, where

Table 1. Ingredient composition of BDs used in the experiments, as-fed basis

	Exp. 1	Exp. 2	
Ingredient, %	BD[1]	BD_{SBM}[2]	BD_{RSC}[3]
Corn	69.51	60.42	60.47
Soybean meal	25.35	35.00	15.00
Rapeseed cake	—	—	20.00
Soybean oil	2.00	2.00	2.00
Minerals and vitamin premix[4]	2.00	2.00	2.00
Limestone	0.41	0.05	—
L-Lysine·H_2SO_4	0.17	—	—
L-Threonine	0.03	—	—
Vitamin E	0.03	0.03	0.03
Titanium dioxide	0.50	0.50	0.50

[1]Corn–soybean meal-based basal diet of experiment 1 (Exp. 1); there were three additional diets intended to have a phytase activity of 750, 1,500, or 3,000 FTU/kg.
[2]Corn–soybean meal-based basal diet of experiment 2 (Exp. 2); there was one additional diet intended to have a phytase activity of 1,500 FTU/kg.
[3]Corn–soybean meal-rapeseed cake-based basal diet of Exp. 2; there was one additional diet intended to have a phytase activity of 1,500 FTU/kg.
[4]P-free mineral and vitamin premix (BASU Mineralfutter GmbH, Bad Sulza, Germany) provided per kg of complete diet: Ca, 4.0 g; Na, 1.0 g; Mg, 200 mg; Fe, 80 mg (iron sulfate); Cu, 10 mg (copper sulfate); Mn, 50 mg (manganese oxide and sulfate); Zn, 60 mg (zinc oxide and sulfate); J, 1.34 mg (calcium iodate); Se, 0.26 mg (sodium selenite); Vitamin A, 7,000 IU; Vitamin D, 1,000 IU; Vitamin E, 50 mg; Vitamin K, 1.0 mg; Vitamin B_1, 1.0 mg: Vitamin B_2, 3.1 mg; Vitamin B_6, 2.5 mg; Vitamin B_{12}, 20 µg; Niacin, 12.5 mg; Pantothenic acid, 8.0 mg; Folic acid, 0.4 mg; Biotin, 0.08 mg; Choline chloride, 160 mg.

additional effects were seen when phytase supplementation was increased from 750 to 1,500 but not to 3,000 FTU/kg.

Both Exp. were arranged as completely randomized Double Latin Square Designs with four diets, eight pigs, and three periods of 12 d each to give six replicates per diet. The 12-d experimental periods consisted of 5 d of adaptation, followed by 5 d for total collection of feces. In Exp. 1, one pig had to be euthanized after surgery due to digestive disorders and could not be replaced. This resulted in five replicates for BD without phytase supplementation, and BD with 1,500 and 3,000 FTU/kg, and six replicates for BD with 750 FTU/kg, respectively.

In Exp. 1, ileal digesta was collected on d 11 from 0715 to 1915 hours and from 1915 hours on day 12 to 0715 hours on day 13. In Exp. 2, digesta was collected on days 11 and 12 from 0715 to 1915 hours each, as no significant differences could be observed in $InsP_6$ hydrolysis comparing day sampling to day and night sampling in Exp. 1 (data not shown). For fecal collection, pigs were fitted with collection bags that were attached to the anus of the pig. Feces were collected to a minimum of two times daily and frozen at –18 °C. Ileal digesta was collected using plastic bags that were attached to the barrel of the cannula with elastic bands. Plastic bags were changed whenever they were filled with digesta, at least every 30 min, and samples were frozen immediately at –18 °C.

Sample preparation and analyses

Feces samples were pooled within pig and period and weighed before analysis. Digesta samples were pooled within pig, period, and sampling interval (day and night for Exp. 1; day 1 and day 2 for Exp. 2), and weighed before the analysis. Samples were freeze-dried, ground through a 0.5-mm sieve and pulverized by a vibrating cup mill (PULVERISETTE 9, Fritsch GmbH, Idar-Oberstein, Germany).

Experimental diets, feces, and ileal digesta were analyzed according to the official methods used in Germany (VDLUFA, 1976) for DM (method 3.1) and CP (method 4.1.1), and for total P, Ca, and Ti as described by Boguhn et al. (2009) and Zeller et al. (2015). Experimental diets were further analyzed for ash (method 8.1), ether extract (**EE**; method 5.1.1 using petroleum ether), and crude fiber (**CF**; method 6.1.1) (VDLUFA, 1976). In SBM and RSC of Exp. 2, CP, EE, P, Ca, neutral detergent fiber (**NDF**), determined without residual ash and after treatment with α-amylase (method 6.5.1), acid detergent fiber (**ADF**), determined without residual ash (method 6.5.2), and acid detergent lignin (**ADL**; method 6.5.3) were analyzed. GE in experimental diets, feces, and ileal digesta was determined using a bomb calorimeter (C 200; Ika-Werke GmbH & Co. KG, Staufen, Germany).

The extraction and measurement of $InsP_{3-6}$ isomers in the experimental diets, feces, and ileal digesta were carried out using the method of Zeller et al. (2015) with slight modifications as described by Sommerfeld et al. (2018b) and measured by high performance ion chromatography (ICS-3000 system, Dionex, Idstein, Germany). Using this methodology, the separation of enantiomers is not possible. Hence, the presentation of results does not distinguish between D- and L-forms. Some $InsP_3$ isomers could not be identified because standards were unavailable. A clear discrimination of the isomers Ins(1,2,6)P_3, Ins(1,4,5)P_3, and Ins(2,4,5)P_3 was not possible because of co-elution; therefore, in the present study, we used the term $InsP_{3x}$ for the $InsP_3$ isomers of unknown proportion. For the analysis of $InsP_{1-2}$ isomers in experimental diets, digesta, and feces, an extraction was performed with a buffer containing 50 mM Tris, 50 mM glycine, and 0.2 M sodium fluoride at pH 9, and otherwise carried out as for $InsP_{3-6}$ isomers. *Myo*-inositol in experimental diets, feces, and ileal digesta samples was analyzed according to the method of Sommerfeld et al. (2018a) using a gas-chromatograph/mass spectrometer after derivatization of the samples.

Experimental diets were analyzed for supplemented phytase activity (AB Vista Laboratories, Innovation & Technology Centre, Ystrad Mynach, UK). Enzyme activity was measured using the analytical method of the enzyme supplier (pH 4.5; 60 °C), and values were converted to FTU by a validated conversion factor. In the unsupplemented BDs, intrinsic plant phytase activity was additionally analyzed using the direct incubation method of Greiner and Egli (2003). In brief, diet samples were incubated in sodium acetate buffer containing 100 µmol sodium phytate at pH 5 and 45 °C. Inorganic phosphate liberated in 20 min was measured spectrophotometrically using ammonium molybdate.

In experimental diets and ileal digesta, AA were analyzed as described by Rodehutscord et al. (2004). Briefly, samples were oxidized and then hydrolyzed at 113 °C for 24 h in a mixture containing HCl and phenol. Norleucine was used as an external standard. The AA were separated and detected using an L-8900 Amino Acid Analyzer (VWR, Hitachi Ltd, Tokyo, Japan). Methionine and cysteine were determined as methionine sulfone and cysteic acid, respectively. The concentrations of tyrosine, histidine, and phenylalanine may be affected by the oxidation procedure (Mason et al., 1980). Tryptophan was determined by using reversed-phase chromatography and fluorescence detection after alkaline hydrolysis using barium hydroxide according to Scheuermann and Eckstein (1986).

Proteolytic enzyme activities were measured in ileal digesta of Exp. 2. Samples were gently thawed on ice and duplicate 200 mg of

digesta was used for each enzyme activity measurements. Trypsin and chymotrypsin enzymatic activities were measured using the Colorimetric Trypsin Activity Assay Kit (ScienCell Research Laboratories, Carlsbad, CA) and Chymotrypsin Activity Assay Kit (BioVision, Inc., Milpitas, CA) following the protocol guidelines. Carboxypeptidase A and B were measured using a 50 mM Tris/100 mM NaCl buffer (pH 7.5). N-(4-Methoxyphenylazoformyl)-Phe-OH potassium salt and N-(4-Methoxyphenylazoformyl)-Arg-OH HCl (Bachem AG, Bubendorf, Switzerland) were used as substrates for carboxypeptidase A and B, respectively. Pure enzymes (Sigma-Aldrich Chemie GmbH, München, Germany; Abnova, Taipei, Taiwan) were used as positive controls and for standard calibration. Kinetic measurements were done at 350 nm for 10 min.

Calculations and statistical analysis

Prececal $InsP_6$ hydrolysis was calculated for each pig based on the analyzed content of $InsP_6$ and Ti in experimental diets and ileal digesta. The following generally accepted equation was used:

$$InsP_6\ hydrolysis\ (\%) = 100 - 100 \times \left(\frac{Ti\ in\ diet}{Ti\ in\ digesta}\right) \times \left(\frac{InsP_6\ in\ digesta}{InsP_6\ in\ diet}\right)$$

where Ti and $InsP_6$ are in g/kg DM. The prececal DM, CP, AA, P, Ca, and GE digestibility and total tract $InsP_6$ hydrolysis and total tract DM, CP, P, Ca, and GE digestibility were calculated accordingly. A correction for endogenous losses was not applied.

The pig was the experimental unit and animal and period were included in the model as random effects. Outliers were identified by Grubbs' test (GraphPad Software, San Diego, CA). In Exp. 1, all data were analyzed in a one-factorial analysis of variance using the MIXED procedure of SAS (version 9.3; SAS Institute Inc., Cary, NC). The experimental diet was the fixed effect. The model was:

$$y_i = \mu + \alpha_i + e_i$$

where y_i = response variable, μ = overall mean, α_i = effect of the experimental diet, and e_i = error term.

In Exp. 2, data were subjected to a two-factorial analysis of variance using the MIXED procedure of SAS. Protein source (SBM or SBM and RSC) and phytase inclusion level (0 or 1,500 FTU/kg), as well as their interaction, were the fixed effects. The model was:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

where y_{ijk} = kth observation of the ith protein source and jth phytase inclusion level, μ = overall mean, α_i = effect of the ith protein source, β_j = effect of the jth phytase inclusion level, $(\alpha\beta)_{ij}$ = interaction effect between the ith protein source and the jth phytase inclusion level, and e_{ijk} = error term.

For both Exp., the assumptions of normality and variance homogeneity of residuals were checked graphically, and, if necessary, subjected to log or square-root transformation. All $InsP_6$ hydrolysis and digestibility data were logit transformed. If the F-test was significant, the multiple t-test was used for treatment comparison. The results are presented via letter-based display. The level of significance was set at $\alpha = 0.05$.

Results

Nutrient composition of diets

The intended concentrations of Ca, total P, and $InsP_6$-P as well as supplemented phytase were confirmed by the analysis (Table 2). For Exp. 2, RSC-containing diets had higher Ca, total P, and $InsP_6$-P concentrations when compared with SBM-based diets. InsP isomers lower than $InsP_5$ were not detected. Intrinsic plant phytase activity of the three unsupplemented BDs was below 100 U/kg (data not shown).

Exp. 1: phytase supplementation level

InsP isomers in ileal digesta and feces

The concentrations of different positional InsP isomers and *myo*-inositol in ileal digesta and feces are presented in Table 3. In the ileal content, InsP isomer concentration significantly differed ($P < 0.01$) for almost all analyzed InsP that were detectable, except for Ins(1,2)P_2. The concentration of $InsP_6$ was greatest ($P < 0.001$) and that of *myo*-inositol lowest ($P < 0.001$) in pigs fed the unsupplemented diet. The ileal digesta concentration of $InsP_6$ decreased by 25.5, 28.8, and 29.5 µmol/kg DM with supplementation of 750, 1,500 and 3,000 FTU/kg diet while the concentration of Ins(1,2,5,6)P_4 increased ($P < 0.001$) by 8.3, 5.6, and 4.6 µmol/g DM, respectively, when compared with the BD. While Ins(1,2)P_2 and $InsP_{3x}$ were not detectable in ileal digesta of pigs fed the BD, 1.7 to 2.9 µmol/g DM of these InsP were analyzed in pigs fed the phytase-supplemented diets. In feces, $InsP_2$, $InsP_3$, and Ins(1,2,3,4,6)P_5 were not detectable in the majority of samples. Phytase supplementation did not significantly affect the concentrations of Ins(1,2,5,6)P_4 and $InsP_6$ in feces. The concentration of *myo*-inositol was greatest ($P = 0.014$) in feces of pigs fed the BD when compared with diets supplemented with 1,500 and 3,000 FTU/kg diet. Concentrations of InsP isomers and *myo*-inositol in feces overall were low.

Prececal and total tract digestibility of dry matter, energy, nitrogen, phosphorus, calcium, and phytate hydrolysis

Prececal digestibility of P and $InsP_6$ hydrolysis were higher ($P < 0.001$) in phytase-supplemented diets but not different between diets supplemented with 1,500 and 3,000 FTU/kg (Table 4). The prececal Ca digestibility was lower ($P = 0.041$) in pigs fed BD compared with the supplemented diets. There were no differences in prececal and total tract digestibility of DM, GE, and N among the treatments. Total tract $InsP_6$ hydrolysis was nearly complete in all pigs. Total tract P digestibility was lower ($P < 0.001$) in pigs fed the BD when compared with the supplemented diets. Total tract Ca digestibility of the BD differed ($P = 0.019$) from supplementation levels 750 and 1,500 FTU/kg but not from the highest supplementation level.

Prececal amino acid digestibility

There was no significant effect of phytase supplementation on prececal AA digestibility (Table 5). Overall, prececal AA digestibility was lowest for Gly with values ranging from 62.1% to 63.7% and greatest for Arg with values ranging from 85.3% to 86.5%.

Exp. 2: protein source and phytase supplementation

Three outliers were detected for $InsP_6$ in feces and values were excluded from statistical analysis. Therefore, number of observations for fecal $InsP_6$ concentration and total tract $InsP_6$ hydrolysis for BD_{SBM} and BD_{RSC} without phytase supplementation were $n = 5$ and $n = 4$, respectively, and for BD_{SBM} and BD_{RSC} with phytase supplementation $n = 6$.

InsP isomers in ileal digesta and feces

Concentrations of InsP isomers in ileal digesta and feces are presented in Table 6. In ileal digesta, an interaction of

Table 2. Analyzed chemical composition of the experimental diets of Exp. 1 and Exp. 2, g/kg DM, if not stated otherwise

	Exp. 1				Exp. 2			
	BD[1]				BD_{SBM}[2]		BD_{RSC}[3]	
Phytase:	0[4]	750	1,500	3,000	0[4]	1,500	0[4]	1,500
Dry matter, g/kg	900	902	901	901	893	894	893	895
Ash	57	56	56	55	60	59	59	58
Ether extract	66	68	65	66	58	58	83	85
Crude protein	187	192	187	188	233	235	199	202
Crude fiber	23	27	28	27	21	21	35	33
Gross energy, MJ/kg DM	19.5	19.6	19.5	19.5	19.2	19.2	19.5	19.6
Calcium	7.0	7.0	6.9	6.8	5.7	5.4	6.8	6.4
Total phosphorus (P)	3.7	3.8	3.7	3.7	4.4	4.5	5.5	5.4
$InsP_6$-P	2.4	2.5	2.4	2.5	2.9	3.0	3.9	3.8
$InsP_6$, µmol/g DM	13.0	13.3	13.1	13.3	15.5	16.1	21.1	20.3
Ins(1,2,3,4,6)P_5, µmol/g DM	<LOQ[5]	<LOQ	<LOQ	<LOQ	0.3	0.3	0.2	0.2
Ins(1,2,3,4,5)P_5, µmol/g DM	0.3	0.4	0.4	0.4	0.6	0.6	0.4	0.4
Ins(1,2,4,5,6)P_5, µmol/g DM	0.9	0.9	0.9	0.9	1.1	1.1	0.8	0.7
myo-Inositol, µmol/g DM	1.6	1.7	1.6	1.7	1.7	1.7	1.1	1.7
Phytase activity (FTU/kg)[6]	<50	792	1,710	3,140	<50	1,530	<50	1,370
Indispensable amino acids								
Arg	11.7	11.8	11.7	11.7	15.7	15.6	12.1	12.1
His	5.5	5.7	5.6	5.6	6.9	6.8	5.8	5.9
Ile	7.7	7.9	7.6	7.6	10.2	10.0	7.9	7.9
Leu	17.0	17.3	17.0	17.1	21.1	20.9	17.3	17.2
Lys	10.4	10.3	10.2	10.4	12.8	12.7	10.5	10.5
Met	3.0	3.0	3.0	3.0	3.6	3.6	3.5	3.5
Phe	9.4	9.6	9.4	9.4	12.0	11.9	9.2	9.2
Thr	7.6	7.8	7.6	7.7	9.4	9.4	8.2	8.2
Trp	2.3	2.4	2.2	2.2	2.9	3.0	2.6	2.6
Val	8.7	8.9	8.6	8.6	11.2	11.1	9.5	9.5
Dispensable amino acids								
Ala	10.2	10.4	10.2	10.3	12.3	12.1	10.4	10.4
Asx[7]	19.0	19.6	19.0	19.1	25.4	25.2	17.9	17.9
Cys	3.0	3.1	3.0	3.0	3.5	3.5	3.8	3.8
Glx[7]	33.8	34.8	34.1	34.3	43.8	43.7	35.9	35.6
Gly	7.9	8.1	7.9	8.0	9.9	9.8	8.8	8.8
Pro	10.8	10.5	10.7	10.6	13.9	13.9	12.6	12.6
Ser	9.6	9.9	9.7	9.9	12.2	12.2	9.6	9.6
Tyr	6.6	6.8	6.6	6.6	8.3	8.2	6.6	6.6

[1]Corn–soybean meal (SBM)-based basal diet of experiment 1 (Exp. 1.).
[2]Corn–SBM-based basal diet of experiment 2 (Exp. 2); analyzed concentration of nutrients in SBM, in % on dry matter (DM) basis: P, 0.75, Ca, 0.51; CP, 51.2; EE, 2.6; NDF, 17.3; ADF, 6.9; ADL, 0.6.
[3]Corn–SBM–rapeseed cake (RSC)-based basal diet of Exp. 2; analyzed concentration of nutrients of RSC, in % on DM basis: P, 1.24; Ca, 1.54; CP, 33.9; EE, 14.9; NDF, 23.0; ADF, 19.2; ADL, 6.8.
[4]Intrinsic plant phytase activity of basal diets was below the detection limit.
[5]Below the limit of quantification.
[6]Determined at pH 4.5 and 60 °C.
[7]Asp, Asn, and Glu, Gln, respectively, were detected together because the side groups of Asn and Gln are lost during acid hydrolysis (Fontaine, 2003).

protein source × phytase supplementation was only found for Ins(1,2,3,4,5)P_5, with a greater phytase supplementation effect in BD_{SBM} than BD_{RSC} fed pigs ($P = 0.017$). Phytase supplementation significantly affected the concentration of all detectable InsP isomers in ileal digesta ($P < 0.001$) by decreasing $InsP_6$ and $InsP_5$, and increasing Ins(1,2,5,6)P_4, $InsP_{3x}$, and Ins(1,2)P_2 concentrations. Ileal *myo*-inositol concentration increased ($P < 0.001$) due to phytase supplementation, but was lower in pigs fed BD_{RSC} compared with BD_{SBM} ($P = 0.004$). In feces, no interactive effect of protein source × phytase supplementation was observed. The concentration of Ins(1,2,4,5,6)P_5 in feces was significantly affected by protein source ($P = 0.045$) and phytase supplementation ($P = 0.003$). In the majority of fecal samples, $InsP_2$, $InsP_3$, Ins(1,2,3,4)P_4, Ins(1,2,3,4,6)P_5, and Ins(1,2,3,4,5)P_5 were not detectable.

Prececal and total tract digestibility of dry matter, energy, nitrogen, phosphorus, calcium, and phytate hydrolysis

No interactive effect was observed on prececal and total tract digestibility of any nutrient and $InsP_6$ hydrolysis (Table 7). Phytase supplementation increased ($P < 0.001$) prececal and total tract digestibility of P and Ca, and prececal hydrolysis of $InsP_6$, whereas there was no effect on DM, GE, and N digestibility. Lower prececal and total tract digestibility values were found for BD_{RSC} than BD_{SBM} ($P < 0.05$), but no difference in the $InsP_6$ hydrolysis. The increase in concentrations of prececal digestible

Table 3. Effects of increasing the inclusion level of phytase on the concentrations of different inositol phosphate (InsP) isomers and *myo*-inositol in ileal digesta and feces, µmol/g DM (Exp. 1)[1]

	BD[2]					
Phytase:	0	750	1.500	3,000	Pooled SEM	*P*-value
Ileal digesta						
$Ins(1,2)P_2$	n.d.[3]	1.8	1.8	1.7	0.4	0.999
$InsP_{3x}$[4]	n.d.	2.9[b]	1.8[a]	1.7[a]	0.3	0.009
$Ins(1,5,6)P_3$	<LOQ[5]	<LOQ	0.2	<LOQ	.	.
$Ins(1,2,3,4)P_4$	0.2	n.d.	n.d.	n.d.	.	.
$Ins(1,2,5,6)P_4$	0.4[a]	8.7[c]	6.0[b]	5.0[b]	0.8	<0.001
$Ins(1,2,3,4,6)P_5$	0.6	n.d.	n.d.	n.d.	.	.
$Ins(1,2,3,4,5)P_5$	1.4[b]	1.2[b]	0.6[a]	0.4[a]	0.1	<0.001
$Ins(1,2,4,5,6)P_5$	2.6[c]	0.6[b]	0.4[a]	0.3[a]	0.1	<0.001
$InsP_6$	36.0[c]	10.5[b]	7.2[a]	6.5[a]	0.8	<0.001
Myo-inositol	1.8[a]	6.3[b]	9.2[c]	9.7[c]	1.1	<0.001
Feces						
$Ins(1,2,5,6)P_4$	n.d.	0.3	n.d.	0.2	0.1	0.250
$Ins(1,2,3,4,5)P_5$	n.d.	0.2	n.d.	n.d.	0.1	.
$Ins(1,2,4,5,6)P_5$	0.4[b]	0.3[ab]	n.d.	0.2[a]	0.1	0.046
$InsP_6$	3.3	2.5	1.5	2.6	0.7	0.178
Myo-inositol	1.2[c]	0.9[bc]	0.4[a]	0.5[ab]	0.2	0.014

[1]Data are given as LSMeans and pooled SEM (untransformed data); InsP not mentioned here were not detectable in ileal digesta and feces. $InsP_1$ isomers were not well separated from co-eluting matrix components and, therefore, not precisely identified. However, concentrations of $InsP_1$ isomers potential including other components were <1.5 µmol/g DM for all samples.
[2]Corn–soybean meal-based basal diet.
[3]n.d., not detectable in the majority of samples.
[4]At least one of the following isomers: $Ins(1,2,6)P_3$, $Ins(1,4,5)P_3$, $Ins(2,4,5)P_3$.
[5]<LOQ, below limit of quantification in the majority of samples.
[a–c]Within a row, means without a common superscript differ ($P < 0.05$).

Table 4. Effects of increasing the inclusion level of phytase on the prececal and total tract digestibility of energy, nitrogen, phosphorus, and calcium, and on the prececal and total tract $InsP_6$ hydrolysis of corn–soybean meal-based diets fed to growing pigs, % (Exp. 1)[1]

	BD[2]					
Phytase:	0	750	1,500	3,000	Pooled SEM	*P*-value
Prececal						
Dry matter	69.8	69.8	69.1	69.9	1.0	0.561
Energy	73.8	73.5	72.7	73.3	0.9	0.319
Nitrogen	73.1	74.2	73.7	74.6	1.6	0.521
Phosphorus	16.3[a]	50.5[b]	60.5[c]	61.4[c]	2.1	<0.001
Calcium	45.0[a]	58.6[b]	62.1[b]	57.5[b]	4.7	0.041
$InsP_6$	18.4[a]	76.3[b]	83.2[c]	85.0[c]	1.5	<0.001
Total tract						
Dry matter	86.9	87.2	87.6	87.1	0.3	0.090
Energy	88.1	87.8	87.9	87.5	0.3	0.369
Nitrogen	84.6	85.3	86.1	85.6	0.7	0.161
Phosphorus	22.7[a]	48.8[b]	58.7[b]	53.9[b]	4.4	<0.001
Calcium	36.0[a]	49.5[b]	59.9[b]	46.8[ab]	5.3	0.019
$InsP_6$	96.6	97.5	98.6	97.4	0.7	0.102

[1]Data are given as LSMeans and pooled SEM (untransformed data).
[2]Corn–soybean meal-based basal diet.
[a–c]Within a row, means without a common superscript differ ($P < 0.05$).

P and $InsP_6$-P due to phytase supplementation amounted to 1.43 and 1.80 g/kg DM for BD_{SBM} and 1.87 and 2.39 g/kg DM for BD_{RSC} (Figure 1). Concentrations of prececal digestible P, calculated as digestibility times concentration in the diet, amounted to 1.31, 2.74, 1.23, and 3.10 g/kg DM and of prececal digestible $InsP_6$-P to 0.92, 2.72, 1.16, and 3.55 g/kg DM in BD_{SBM} without and with phytase and BD_{RSC} without and with phytase, respectively. The response to phytase in the amount of digestible P was about 20% lower than the response in $InsP_6$-P hydrolysis independent of the protein source (Figure 1). This mirrors the amount of P bound in lower InsP at the end of the ileum.

Prececal amino acid digestibility

There was no interactive effect of protein source × phytase supplementation on prececal AA digestibility (Table 8). However, digestibility of most AA, except for Cys and Glx, was lower in

Table 5. Effects of increasing the inclusion level of phytase on the apparent prececal amino acid digestibility of corn–soybean meal-based diets fed to growing pigs, % (Exp. 1)[1]

	BD[2]					
Phytase:	0	750	1,500	3,000	Pooled SEM	*P*-value
Indispensable amino acids						
Arg	85.3	86.2	86.4	86.5	1.0	0.291
His	75.3	76.2	75.4	76.3	1.6	0.662
Ile	79.2	80.6	79.9	80.1	1.6	0.561
Leu	79.8	80.9	80.3	81.3	1.7	0.453
Lys	78.7	79.1	79.8	80.4	1.5	0.440
Met	81.3	82.0	81.8	81.7	1.7	0.928
Phe	80.1	81.6	81.2	81.8	1.5	0.283
Thr	67.0	68.3	67.3	68.3	1.8	0.628
Trp	70.5	71.6	68.4	69.4	1.9	0.223
Val	75.1	76.3	75.0	75.8	1.9	0.646
Dispensable amino acids						
Ala	73.5	74.6	74.2	75.1	2.1	0.681
Asx[3]	73.8	75.4	75.2	76.3	1.6	0.165
Cys	66.1	66.9	65.0	66.3	1.8	0.551
Glx[3]	80.4	80.7	81.3	82.1	1.4	0.342
Gly	62.7	63.1	62.1	63.7	1.9	0.869
Pro	76.4	76.0	76.6	75.8	1.7	0.913
Ser	73.5	74.9	74.6	75.9	1.7	0.171
Tyr	79.2	80.5	79.5	80.3	1.5	0.455

[1]Data are given as LSMeans and pooled SEM (untransformed data).
[2]Corn–soybean meal-based basal diet.
[3]Asp, Asn, and Glu, Gln, respectively, were detected together because the side groups of Asn and Gln are lost during acid hydrolysis (Fontaine, 2003).

Table 6. Concentrations of inositol phosphate (InsP) isomers and *myo*-inositol in ileal digesta and feces of pigs fed corn–soybean meal- or corn–soybean meal–rapeseed cake-based diets without or with the supplementation of phytase, µmol/g DM (Exp. 2)[1]

	BD_{SBM}[2]		BD_{RSC}[3]			*P*-value		
Phytase:	0	1,500	0	1,500	SEM	Protein source	Phytase	Protein source × phytase
Ileal digesta								
$Ins(1,2)P_2$	n.d.[4]	5.0	n.d.	5.3	0.7	0.985	.	.
$InsP_{3x}$[5]	n.d.	4.7	0.8	4.3	0.5	0.587	< 0.001	.
$Ins(1,2,3,4)P_4$	0.3	n.d.	0.5	n.d.	0.1	< 0.001	.	.
$Ins(1,2,5,6)P_4$	0.4	8.4	0.8	8.3	1.2	0.664	< 0.001	0.867
$Ins(1,2,3,4,6)P_5$	0.5	n.d.	<LOQ[6]	n.d.	0.0	.	.	.
$Ins(1,2,3,4,5)P_5$	1.2[a]	0.4[c]	1.0[ab]	0.8[b]	0.1	0.379	< 0.001	0.017
$Ins(1,2,4,5,6)P_5$	3.0	0.2	2.7	0.3	0.1	0.944	< 0.001	0.050
$InsP_6$	32.2	3.9	43.2	4.7	1.9	0.028	< 0.001	0.565
Myo-inositol	4.7	17.7	2.7	13.2	1.6	0.004	< 0.001	0.787
Feces								
$Ins(1,2,5,6)P_4$	0.5	n.d.	0.5	0.3	0.2	0.809	0.263	.
$Ins(1,2,4,5,6)P_5$	0.7	n.d.	1.5	0.3	0.4	0.045	0.003	.
$InsP_6$[7]	1.3	1.3	1.8	1.3	0.4	0.430	0.364	0.875
Myo-inositol	0.3	0.5	0.6	<LOQ	0.1	0.187	0.324	.

[1]Data are given as LSMeans and pooled SEM (untransformed data); InsP not mentioned here were not detectable. $InsP_1$ isomers were not well separated from co-eluting matrix components and, therefore, not precisely identified. However, concentrations of $InsP_1$ isomers potential including other components were < 1.5 µmol/g DM for all samples.
[2]Corn–soybean meal-based basal diet.
[3]Corn–soybean meal-rapeseed cake-based basal diet.
[4]n.d., not detectable in the majority of samples.
[5]At least one of the following isomers: $Ins(1,2,6)P_3$, $Ins(1,4,5)P_3$, $Ins(2,4,5)P_3$.
[6]<LOQ, below limit of quantification in the majority of samples.
[7]Number of observations for BD_{SBM} and BD_{RSC} without phytase supplementation were $n = 5$ and $n = 4$, respectively, and for BD_{SBM} and BD_{RSC} with phytase supplementation were $n = 6$, respectively.
[a–c]Means in a row not sharing a common superscript differ significantly (multiple *t*-tests in case of interaction) ($P < 0.05$).

Table 7. Prececal and total tract digestibility of energy, nitrogen, phosphorus, and calcium, and prececal and total tract $InsP_6$ hydrolysis of corn–soybean meal- or corn–soybean meal–rapeseed cake-based diets without or with the supplementation of phytase, % (Exp. 2)[1]

	BD_{SBM}[2]		BD_{RSC}[3]			P-value		
Phytase:	0	1,500	0	1,500	SEM	Protein source	Phytase	Protein source × phytase
Prececal								
Dry matter	67.2	67.4	65.6	66.1	1.1	0.013	0.472	0.863
Energy	70.5	70.3	68.4	68.6	1.1	0.004	0.982	0.719
Nitrogen	77.8	77.6	70.8	72.1	0.8	<0.001	0.438	0.276
Phosphorus	29.4	61.5	22.5	56.9	2.3	0.017	<0.001	0.376
Calcium	54.9	68.5	47.0	63.4	2.8	0.020	<0.001	0.772
$InsP_6$	31.2	92.1	30.1	92.3	2.5	0.360	<0.001	0.600
Total tract								
Dry matter	86.9	86.9	82.2	83.2	0.5	<0.001	0.083	0.062
Energy	87.5	86.8	82.8	82.9	0.5	<0.001	0.166	0.133
Nitrogen	88.5	88.8	82.0	82.4	1.0	<0.001	0.827	0.923
Phosphorus	33.2	64.3	24.0	51.9	3.2	<0.001	<0.001	0.828
Calcium	49.7	67.5	41.7	57.6	4.3	0.007	<0.001	0.654
$InsP_6$[4]	98.9	98.9	98.5	98.9	0.4	0.607	0.552	0.687

[1]Data are given as LSMeans and pooled SEM (untransformed data).
[2]Corn–soybean meal-based basal diet.
[3]Corn–soybean meal–rapeseed cake-based basal diet.
[4]Number of observations for BD_{SBM} and BD_{RSC} without phytase supplementation were $n = 5$ and $n = 4$, respectively, and for BD_{SBM} and BD_{RSC} with phytase supplementation were $n = 6$, respectively.

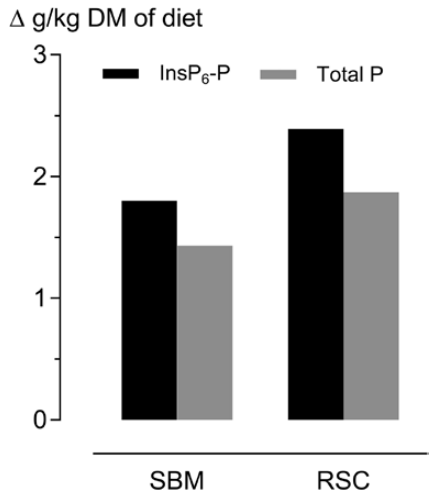

Figure 1. Increase in concentrations of prececal digestible P and hydrolyzed $InsP_6$-P due to phytase supplementation (1,500 FTU/kg) in diets with either soybean meal (SBM) or a mix of SBM–rapeseed cake (RSC) as the main protein sources in Exp. 2. Columns represent the respective differences in concentrations between the diets supplemented with 1,500 and 0 FTU/kg phytase.

BD_{RSC} than BD_{SBM} ($P < 0.05$). On average of indispensable AA, digestibility was 6.6%-points lower in the diet that contained RSC. Phytase supplementation had no effect on prececal AA digestibility.

Enzyme activity in ileal digesta

Enzyme activity in ileal digesta is presented in Table 9. For carboxypeptidase A and trypsin, one outlier each was detected and values were excluded from statistical analysis. For trypsin an interaction of protein source × phytase supplementation was found ($P = 0.042$). Chymotrypsin activity was greater in SBM-based diets when compared with RSC containing diets ($P = 0.010$). The calculated SEM of enzyme activity was overall high.

Discussion

InsPs and *myo*-inositol

Following the initial Exp. of Kemme et al. (2006), some studies were conducted to investigate the presence of $InsP_5$, $InsP_4$, and $InsP_3$ in the digesta of pigs (Blaabjerg et al., 2011; Kühn et al., 2016; Laird et al., 2016, 2018; Mesina et al., 2019). However, except for Kemme et al. (2006), authors did not differentiate positional InsP isomers and Mesina et al. (2019) were the only authors feeding diets including rapeseed.

In both Exp. of the current study, the dominating InsP isomers beside $InsP_6$ in ileal digesta of pigs fed the BDs were Ins(1,2,3,4,5)P_5 and Ins(1,2,4,5,6)P_5. Their proportions in $\Sigma InsP_{1\text{-}5}$ were 0.77 and 0.78 for SBM-based BDs for Exp. 1 and Exp. 2, respectively, and 0.64 for BD_{RSC} of Exp. 2. The $InsP_5$ appearance might have been caused by intrinsic plant phytases, phytase-producing microbes in the feed, or microbial phytases of the gastrointestinal tract (Zeller et al., 2015). The proportion of $InsP_4$ isomers in $\Sigma InsP_{1\text{-}5}$ was low in BDs (0.12 and 0.13 for SBM-based BDs of Exp. 1 and 2, respectively, and 0.22 in the SBM-RSC-based BD of Exp. 2). However, ileal InsP concentration pattern remarkably changed after the supplementation of microbial phytase. In Exp. 1, the proportion of $\Sigma InsP_5$ isomers in ileal $\Sigma InsP_{1\text{-}5}$ decreased sharply when phytase was supplemented (0.12, 0.09, and 0.08 $\Sigma InsP_5$ in $\Sigma InsP_{1\text{-}5}$ for supplementation levels 750, 1,500, and 3,000 FTU/kg diet, respectively). Furthermore, the Ins(1,2,5,6)P_4 concentration increased markedly (proportion in $\Sigma InsP_{1\text{-}5}$: 0.57, 0.56, and 0.55 for supplementation levels 750, 1,500, and 3,000 FTU/kg diet) compared with the BD (0.12). Ins(1,2,5,6)P_4 is a typical isomer found in the degradation pathway of a 6-phytase and not differentiated herein from its enantiomer Ins(2,3,4,5)P_4 as this is co-eluted during the analysis (Greiner and Konietzny, 2011). These results are consistent with the study of Mesina et al. (2019) that showed supplementation of 750, 1,500, and 3,000 FTU/kg diet to a corn–SBM–rapseed meal (**RSM**) diet leads to a proportion of $\Sigma InsP_4$ in $\Sigma InsP_{3\text{-}5}$ of 0.58, 0.64, and 0.55. This increase in $InsP_4$ fraction relative to other InsP in ileal digesta of pigs is in accordance with results presented by Kühn et al. (2016)

Table 8. Apparent prececal AA digestibility in corn–soybean meal- or corn–soybean meal–rapeseed cake-based basal diet without or with the supplementation of phytase, % (Exp. 2)[1]

	BD_{SBM}[2]		BD_{RSC}[3]			*P*-value		
Phytase:	0	1,500	0	1,500	SEM	Protein source	Phytase	Protein source × phytase
Indispensable amino acids								
Arg	89.2	89.1	83.2	84.4	0.5	<0.001	0.177	0.131
His	81.5	80.2	75.3	75.8	0.9	<0.001	0.448	0.191
Ile	85.3	84.6	76.1	77.1	0.8	<0.001	0.939	0.259
Leu	84.7	84.0	78.4	78.9	0.8	<0.001	0.774	0.348
Lys	82.4	81.5	74.7	75.9	0.8	<0.001	0.992	0.130
Met	84.9	84.2	81.1	81.2	1.0	0.001	0.654	0.603
Phe	85.5	85.3	77.9	79.1	0.7	<0.001	0.478	0.288
Thr	74.9	73.8	65.2	65.7	1.2	<0.001	0.684	0.432
Trp	77.3	76.4	68.8	70.5	1.2	<0.001	0.750	0.219
Val	82.1	80.9	72.8	73.6	0.9	<0.001	0.638	0.201
Dispensable amino acids								
Ala	79.0	78.1	72.5	73.0	1.2	<0.001	0.633	0.396
Asx[4]	79.6	79.5	72.1	73.1	0.8	<0.001	0.523	0.403
Cys	71.2	67.8	70.0	70.5	1.4	0.582	0.280	0.142
Glx[4]	83.0	83.0	81.3	82.5	0.9	0.070	0.362	0.426
Gly	69.4	68.8	61.9	63.3	1.2	<0.001	0.604	0.172
Pro	78.2	78.3	74.1	75.3	1.6	0.003	0.676	0.549
Ser	80.4	80.1	70.7	71.5	1.0	<0.001	0.829	0.551
Tyr	84.3	83.6	75.3	75.7	1.0	<0.001	0.674	0.419

[1]Data are given as LSMeans and pooled SEM (untransformed data).
[2]Corn–soybean meal-based basal diet.
[3]Corn–soybean meal–rapeseed cake-based basal diet.
[4]Asp, Asn, and Glu, Gln, respectively, were detected together because the side groups of Asn and Gln are lost during acid hydrolysis (Fontaine, 2003).

Table 9. Enzyme activities in ileal digesta of pigs fed corn–soybean meal- or corn–soybean meal–rapeseed cake-based diets without or with the supplementation of phytase, nmol/min/g (Exp. 2)[1]

	BD_{SBM}[2]		BD_{RSC}[3]			*P*-value		
Phytase:	0	1,500	0	1,500	SEM	Protein source	Phytase	Protein source × phytase
Carboxypeptidase A[4]	1,156	1,433	1,064	1,449	299	0.600	0.704	0.495
Carboxypeptidase B	3,824	3,762	3,489	2,489	570	0.102	0.244	0.432
Trypsin[5]	195[a]	257[ab]	300[b]	245[ab]	37	0.054	0.865	0.042
Chymotrypsin	19	22	16	14	2	0.010	0.783	0.184

[1]Data are given as LSMeans and pooled SEM (untransformed data).
[2]Corn–soybean meal-based basal diet.
[3]Corn–soybean meal–rapeseed cake-based basal diet.
[4]Number of observations for unsupplemented diets and supplemented BD_{RSC} were $n = 6$ each, and for BD_{SBM} with phytase supplementation $n = 5$.
[5]Number of observations for supplemented diets and unsupplemented BD_{RSC} were $n = 6$ each, and for BD_{SBM} without phytase supplementation $n = 5$.
[a–b]Means in a row not sharing a common superscript differ significantly (multiple *t*-tests in case of interaction) ($P < 0.05$).

and Laird et al. (2016), whereas such an increase could not be observed by Laird et al. (2018). However, all of these authors did not distinguish between single positional InsP isomers. For $InsP_{3x}$, greatest ileal concentrations were observed with 750 FTU/kg diet, demonstrating that beyond this level still further degradation is achieved. Contents of $Ins(1,2)P_2$ and *myo*-inositol also increased due to phytase supplementation in the present study. However, for most InsP isomers, the major change in concentration occurred until the supplementation of 1,500 FTU/kg diet whereas an increase to 3,000 FTU/kg diet did not change the pattern any further. In Exp. 2, the inclusion of 1,500 FTU phytase/kg of diet overcame the differences in InsP pattern between unsupplemented BD_{SBM} and BD_{RSC}, resulting in nearly the same InsP pattern in ileal digesta of pigs fed those diets including 1,500 FTU/kg. With the approach chosen in Exp. 2, the concentration of $InsP_6$ differed between BD_{SBM} and BD_{RSC} because the $InsP_6$ concentration was higher in RSC than SBM. Next to the differences in composition of RSC and SBM, the differences in the supply of substrate per se might have influenced the response to phytase. However, based on results of Exp. 1, the differences in $InsP_6$ concentration are unlikely to be relevant at the chosen supplementation level of 1,500 FTU/kg.

In the hindgut, endogenous phytases most likely of bacterial origin hydrolyze large amounts of $InsP_6$ that left the ileum undegraded. In the present study, this led to very low concentrations of $InsP_{4-6}$ and no $InsP_{1-3}$ detectable in feces,

especially when phytase was supplemented to the diets. However, P liberated in the hindgut is not absorbed (Seynaeve et al., 2000; Schlemmer et al., 2001; Rodehutscord and Rosenfelder, 2016). Hence, though hindgut fermentation resulted in a nearly complete $InsP_6$ hydrolysis, total tract P digestibility did not exceed 65% in the present study.

Ileal *myo*-inositol concentration increased upon phytase supplementation, showing that some of the $InsP_6$ in the diet was completely dephosphorylated. While this is consistent with other studies in pigs (Kühn et al., 2016; Laird et al., 2018; Mesina et al., 2019), it is not consistent with the viewpoint of free inositol being absorbed from the digestive tract of humans with high efficiency (Kohlmeier, 2003) and almost completely (Croze and Soulage, 2013). If all *myo*-inositol released in the digestive tract had been absorbed, then ileal *myo*-inositol concentrations should not have changed. It is not possible to estimate the rate of absorption of released *myo*-inositol from the present study other than that is was not complete. A certain proportion of released *myo*-inositol, however, should have been absorbed, because studies in humans, pigs, and broiler chickens showed an increase in blood plasma *myo*-inositol concentration upon supplementation of free *myo*-inositol (Clements and Reynerston, 1977; Guggenbuhl et al., 2016; Sommerfeld et al., 2018a). In the hindgut, non-absorbed *myo*-inositol likely is nearly degraded by the microbiota or absorbed as indicated by the low fecal *myo*-inositol concentrations in the present study.

Calcium and phosphorus

The P digestibility values of the unsupplemented diets in both Exp. confirm the often reported low ability of pigs to degrade $InsP_6$ and digest P of plant origin (Rodehutscord and Rosenfelder, 2016). When phytase was supplemented, more than 90% of $InsP_6$ was found hydrolyzed in ileal digesta of pigs in Exp. 1 and 85% in Exp. 2. In feces, almost all $InsP_6$ was found hydrolyzed in both Exp. As P digestibility was not further increased from the prececal to the fecal level, the released P in the hindgut could not be utilized by the animal and was excreted in other forms than $InsP_6$.

In both diets of Exp. 2, phytase supplementation led to an increase in the dietary concentration of digestible P that was only about 80% of the increase in hydrolyzed $InsP_6$-P (Figure 1). This reflects the amount of phosphate that remains bound in lower InsP as seen in Table 6. This amount of phosphate present as lower InsP may explain why a recent meta-analysis found that average total tract P digestibility at high phytase inclusion was only 65% (Rosenfelder-Kuon et al., 2020). Phytase supplementation effects were similar in both diets of Exp. 2, demonstrating that the accessibility of $InsP_6$ in theses protein sources by added phytase was the same with 1,500 FTU/kg phytase being used.

Amino acids, nitrogen, and energy

Phytate might form complexes with dietary protein and AA and increase endogenous losses of AA (Zouaoui et al., 2018), resulting in decreased AA digestibility values. The literature is inconsistent in regard to the effect of microbial phytase supplementation on prececal AA digestibility in pigs. In some studies, phytase supplementation increased prececal AA digestibility (Kemme et al., 1999; Almeida et al., 2013; Adedokun et al., 2015), in others not (Liao et al., 2005; Pomar et al., 2008; She et al., 2018; Mesina et al., 2019). Feeding regime, dietary composition, sample collection method, age of pigs, phytase source, and phytase level might be reasons for differences among studies. Results of the present study do not support the view of phytase supplementation affecting AA digestibility, even when SBM is partially replaced by the less digestible RSC. The overall greater AA digestibility in BD_{SBM} when compared with BD_{RSC} might be caused by the higher fiber content in RSC and correspondingly more fiber-bound protein (Table 2). Higher fiber contents in RSC might also be the reason for lower prececal and total tract digestibility of N and GE in RSC-based diets when compared with SBM-based diets in Exp. 2.

Enzyme activity

Phytate may inhibit the activity of endogenous digestive enzymes (Bye et al., 2013), such as trypsin and chymotrypsin (Singh and Krikorian, 1982; Deshpande and Damodaran, 1989). Bye et al. (2013) summarized that protein–phytate complexes are formed because of salt-like linkages (electrostatic interactions) between negatively charged $InsP_6$ and the positively charged basic AA residues of Arg, Lys, and His. Furthermore, it is possible that $InsP_6$ with its multiple binding sites, might be an effective chelator for the cations of metalloenzymes such as carbohydrases (Martin and Evans, 1989). Phytate could also have a negative effect on digestive enzymes as it might limit the bioavailability of Ca. The autocatalytic conversion of trypsinogen to trypsin is Ca dependent. Furthermore, lower trypsin activity limits the activation of other digestive enzymes such as chymotrypsin. Higher chymotrypsin activity in the SBM-based diets when compared with RSC-based diet contradict the results of Valette et al. (1992) who analyzed greater chymotrypsin activity in a RSM-based diet than a casein containing diet of higher protein digestibility.

In the current study, the inclusion of phytase had no effect on these ileal enzyme activities, maybe due to the fact that the precipitation of trypsin and chymotrypsin by $InsP_6$ occurs at pH 3 (Deshpande and Damodaran, 1989) and the pH in the ileum is higher. Furthermore, the amount of "free" $InsP_6$ available to inhibit the digestive enzymes may be relevant as most studies were conducted with in vitro enzyme inhibition studies. "Natural" phytic acid is a highly unstable molecule and always present as a salt of Ca, magnesium, or K (Deshpande and Damodaran, 1989). As the Ca content of the diets met the requirements of pigs, Ca likely was not a limiting factor in the intestine for trypsinogen to be activated. Ileal trypsin activity was lower in unsupplemented SBM-based diet when compared with RSM-based diet, maybe due to higher contents of trypsin inhibitors in the SBM-based diet (Kaewtapee et al., 2018). However, there were no differences in ileal trypsin activity in supplemented diets.

Conclusion

The phytase supplementation increased P digestibility and resulted in very high prececal and almost complete total tract $InsP_6$ hydrolysis. However, total tract P digestibility with high level of phytase supplementation hardly exceeded a value of 60%. The $Ins(1,2,5,6)P_4$ isomer is the main bottleneck in the degradation pathway of InsP for the added phytase used in the present study. A further increase in P digestibility may be possible by research on the removal of this bottleneck. Neither in SBM- nor in SBM-RSC-based diets the inclusion of phytase had an effect on prececal AA digestibility.

Acknowledgments

We would like to thank D. Berghaus, W. Siegert, H. Trapp, and L. Uhland for their help during the animal experiments.

Appreciation is also expressed to M. Steffl and N. Nautscher for surgery of the pigs.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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