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 (InsP6) 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 InsP6 were InsP5 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)P4 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 InsP6 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 InsP6 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 InsP6 degradation in the hindgut.

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

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Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| AA           | amino acid  |
| ADF          | acid detergent fiber |
| ADL          | acid detergent lignin |
| BD           | basal diet |
| BD<sub>SBM</sub> | basal diet based on corn and soybean meal |
| BD<sub>RSC</sub> | 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<sub>6</sub> | 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)) and its salts are the major storage form of P. Pigs are known to be nearly unable to hydrolyze InsP<sub>6</sub> 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<sub>6</sub> recovery in feces of pigs is very low (Baumgärtel et al., 2008; Rutherford et al., 2014). There is a scarcity of information on the stepwise degradation pathway of InsP<sub>6</sub> to lower inositol phosphate (InsP<sub>n</sub>) isomers in the gastrointestinal tract of pigs (Rodehutscord and Rosenfelder, 2016). Furthermore, InsP<sub>6</sub> 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<sub>6</sub> than SBM. Until now, no studies were conducted to compare SBM and RSC as protein sources considering InsP<sub>6</sub> hydrolysis and the concentration of lower InsP in the gastrointestinal tract of pigs. Anti-nutritional effects of InsP<sub>n</sub>, 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<sub>6</sub> 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<sub>6</sub> 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<sub>6</sub> as protein sources were investigated. The preecal digestibility of AA and preecal 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<sup>0.73</sup> 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 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. 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

| Ingredient, % | Exp. 1 BD¹ | Exp. 1 BD₂ | Exp. 1 BD₃ | Exp. 2 BD¹ | Exp. 2 BD₂ | Exp. 2 BD₃ |
|---------------|------------|------------|------------|------------|------------|------------|
| Corn          | 69.51      | 60.42      | 60.47      | 69.51      | 60.42      | 60.47      |
| Soybean meal  | 25.35      | 35.00      | 15.00      | 25.35      | 35.00      | 15.00      |
| Rapseed cake  | —          | —          | 20.00      | —          | —          | 20.00      |
| Soybean oil   | 2.00       | 2.00       | 2.00       | 2.00       | 2.00       | 2.00       |
| Minerals and vitamin premix³ | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Limestone     | 0.41       | 0.05       | —          | 0.17       | —          | —          |
| L-lysine-H₂SO₄ | 0.17       | —          | —          | 0.03       | —          | —          |
| L-Threonine   | 0.03       | —          | —          | 0.03       | —          | —          |
| Vitamin E     | 0.03       | 0.03       | 0.03       | 0.03       | 0.03       | 0.03       |
| Titanium dioxide | 0.50      | 0.50       | 0.50       | 0.50       | 0.50       | 0.50       |

¹Corn–soybean meal-based 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.
²Corn–soybean meal-based diet of experiment 2 (Exp. 2); there was an additional diet intended to have a phytase activity of 1,500 FTU/kg.
³Corn–soybean meal–rapeseed cake–based diet of Exp. 2; there was an additional diet intended to have a phytase activity of 1,500 FTU/kg.
⁴phytase-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); Cu, 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 mg; Vitamin B₁₂, 1.0 mg; Vitamin B₃, 3.1 mg; Vitamin B₆, 2.5 mg; Vitamin B₉, 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 per 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 0715 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 InsP₃ 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 InsP₃ isomers in the experimental diets, feces, and ileal digesta was 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 InsP₃ isomers could not be identified because standards were unavailable. A clear discrimination of the isomers Ins(1,2,6)P₃, Ins(1,4,5)P₃, and Ins(2,4,5)P₃ was not possible because of co-elution; therefore, in the present study, we used the term InsP₃ for the InsP₃ isomers of unknown proportion. For the analysis of InsP₃ 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 InsP₃ 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). Briefly, 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 determined by using reversed-phase chromatography and fluorescence detection after alkaline hydrolysis using barium hydroxide according to Schueermann and Eckstein (1986).
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:

$$\text{InsP}_6 \text{ hydrolysis} (\%) = 100 - 100 \times \left( \frac{\text{Ti in diet}}{\text{Ti in digesta}} \right) \times \left( \frac{\text{InsP}_6 \text{ in diet}}{\text{InsP}_6 \text{ in digesta}} \right)$$

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, $\mu$ = overall mean, $\alpha_i$ = 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 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, $\mu$ = overall mean, $\alpha_i$ = effect of the ith protein source, $\beta_j$ = 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 log 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$_6$ isomers lower than InsP$_6$ 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$_6$ 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$_6$ isomer concentration significantly differed ($P < 0.01$) for almost all analyzed InsP that were detectable, except for Ins(1,2)P$_4$. 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$_4$ and InsP$_6$, 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$_6$, InsP$_n$, 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$_6$ 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$_{sam}$ and BD$_{soc}$ without phytase supplementation were n = 5 and n = 4, respectively, and for BD$_{sam}$ and BD$_{soc}$ with phytase supplementation n = 6.

InsP$_6$ isomers in ileal digesta and feces
Concentrations of InsP$_6$ isomers in ileal digesta and feces are presented in Table 6. In ileal digesta, an interaction of
protein source × phytase supplementation was only found for Ins(1,2,3,4,5)P₅, with a greater phytase supplementation effect in BDSBM 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 InsP₆ and InsP₅, and increasing Ins(1,2,5,6)P₄, InsP₃x, and Ins(1,2)P₂ concentrations. Ileal myo-inositol concentration increased (P < 0.001) due to phytase supplementation, but was lower in pigs fed BDRSC compared with BD SBM (P = 0.004). In feces, no interactive effect of protein source × phytase supplementation was observed. The concentration of Ins(1,2,3,4,5)P₅ in feces was significantly affected by protein source (P = 0.045) and phytase supplementation (P = 0.003). In the majority of fecal samples, 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 |
|--------|--------|
| BD¹ | BD₃₂ | BD₃₃ |
| Phytase: | 0⁴ | 750 | 1,500 | 3,000 | 0⁴ | 1,500 | 0⁴ | 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 |
| InsP₆ | 2.4 | 2.5 | 2.4 | 2.5 | 2.9 | 3.0 | 3.9 | 3.8 |
| InsP₅, 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)P₄, µmol/g DM | <LOQ | <LOQ | <LOQ | <LOQ | 0.3 | 0.3 | 0.2 | 0.2 |
| Ins(1,2,3,4,5)P₅, 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)P₅, µmol/g DM | 0.6 | 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 | 2.2 | 2.2 | 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 | 3.0 | 3.1 | 3.0 | 3.0 | 3.5 | 3.5 | 3.8 | 3.8 |
| Cys | 19.0 | 19.6 | 19.0 | 19.1 | 25.4 | 25.2 | 17.9 | 17.9 |
| Gly | 33.8 | 34.8 | 34.1 | 34.3 | 43.8 | 43.7 | 35.9 | 35.6 |
| Glx | 7.9 | 8.1 | 7.9 | 8.0 | 9.9 | 9.8 | 8.8 | 8.8 |
| Pro | 33.8 | 34.8 | 34.1 | 34.3 | 43.8 | 43.7 | 35.9 | 35.6 |
| Ser | 8.7 | 8.9 | 8.6 | 8.6 | 11.2 | 11.1 | 9.5 | 9.5 |
| Tyr | 6.6 | 6.8 | 6.6 | 6.6 | 8.3 | 8.2 | 6.6 | 6.6 |

¹Corn–soybean meal (SBM)-based basal diet of experiment 1 (Exp. 1).
²Corn–SBM-based basal diet of experiment 2 (Exp. 2).
³Corn–SBM–rapeseed cake (RSC)-based basal diet of Exp. 2.
⁴Intrinsic plant phytase activity of basal diets was below the detection limit.
⁵Below the limit of quantification.
⁶Determined at pH 4.5 and 60 °C.
⁷Asp, Asn, and Glu, Gln, respectively, were detected together because the side groups of Asn and Gln are lost during acid hydrolysis (Fontaine, 2003).
P and InsP$_6$-P due to phytase supplementation amounted to 1.43 and 1.80 g/kg DM for BD$_{basal}$ 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$_{basal}$ 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 BD$_{RSC}$ without and with phytase compared to BD$_{basal}$ without and with phytase, respectively.

**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)**

| Phytase:       | 0    | 750  | 1.500 | 3,000 | Pooled SEM | P-value |
|----------------|------|------|-------|-------|------------|---------|
| Ileal digesta  |      |      |       |       |            |         |
| Ins(1,2)P$_6$  | n.d.$^3$ | 1.8 | 1.8 | 1.7 | 0.4 | 0.999 |
| InsP$_3$      | n.d.$^3$ | 2.9$^a$ | 1.8$^a$ | 1.7$^a$ | 0.3 | 0.009 |
| Ins(1,5,6,8)P$_4$ | <LOQ | <LOQ | 0.2 | <LOQ | . | . |
| Ins(1,2,5,6)P$_4$ | 0.2 | 8.7$^b$ | 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$^a$ | 1.2$^a$ | 0.6$^a$ | 0.4$^a$ | 0.1 | <0.001 |
| Ins(1,2,4,5,6)P$_6$ | 2.6$^b$ | 0.6$^b$ | 0.4$^a$ | 0.3$^a$ | 0.1 | <0.001 |
| InsP$_6$      | 36.0$^b$ | 10.5$^b$ | 7.2$^b$ | 6.5$^b$ | 0.8 | <0.001 |
| Myo-inositol  | 1.8$^a$ | 6.3$^a$ | 9.2$^b$ | 9.7$^b$ | 1.1 | <0.001 |
| Feces         |      |      |       |       |            |         |
| Ins(1,2,5,6)P$_6$ | n.d.$^3$ | 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$_6$ | 0.4$^a$ | 0.3$^{a,b}$ | 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$^a$ | 0.9$^{a,b}$ | n.d. | 0.4$^a$ | 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$_3$ isomers were not well separated from co-eluting matrix components and, therefore, not precisely identified. However, concentrations of InsP$_3$ 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.

$^6$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)**

| 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$^a$ | 60.5$^a$ | 61.4$^a$ | 2.1 | <0.001 |
| Calcium        | 45.0$^b$ | 58.6$^b$ | 62.1$^b$ | 57.5$^b$ | 4.7 | 0.041 |
| InsP$_6$       | 18.4$^a$ | 76.3$^b$ | 83.2$^b$ | 85.0$^b$ | 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$^a$ | 58.7$^a$ | 53.9$^a$ | 4.4 | <0.001 |
| Calcium        | 36.0$^b$ | 49.5$^b$ | 59.9$^b$ | 46.8$^{a,b}$ | 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.

$^a,b$Within a row, means without a common superscript differ (P < 0.05).
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

| BD2 | 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 |
| Asx1 | | 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 |
| Glx3 | | 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 |

1Data are given as LSMeans and pooled SEM (untransformed data).
2Corn–soybean meal-based basal diet.
3Asp, 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

| Protein source | Phytase | Protein source | Phytase | Protein source | Phytase | Protein source | Phytase |
|---------------|---------|---------------|---------|---------------|---------|---------------|---------|
| Phytase: 0 1,500 | 0 1,500 | SEM | Protein source | Phytase | Protein source | Phytase |
| Ileal digesta | | | | | | | |
| Ins(1,2)P2 | n.d.4 | 5.0 | n.d. | 5.3 | 0.7 | 0.985 | . |
| InsP1 | n.d. | 4.7 | 0.8 | 4.3 | 0.5 | 0.587 | < 0.001 |
| Ins(1,2,3,4)P4 | 0.3 | n.d. | 0.5 | n.d. | 0.1 | < 0.001 | . |
| Ins(1,2,5,6)P5 | 0.4 | 8.4 | 0.8 | 8.3 | 1.2 | 0.664 | < 0.001 | 0.867 |
| Ins(1,2,3,4,5,6)P6 | 0.5 | n.d. | <LOQ6 | n.d. | 0.0 | . |
| Ins(1,2,3,4,5)P5 | 1.2a | 0.4c | 1.0bc | 0.8a | 0.1 | 0.379 | < 0.001 | 0.017 |
| InsP1 | 3.0 | 0.2 | 2.7 | 0.3 | 0.1 | 0.944 | < 0.001 | 0.050 |
| Myo-inositol | 32.2 | 3.9 | 43.2 | 4.7 | 1.9 | 0.028 | < 0.001 | 0.565 |
| Feces | | | | | | | |
| Ins(1,2,5,6)P4 | 0.5 | n.d. | 0.5 | 0.3 | 0.2 | 0.809 | 0.263 | . |
| Ins(1,2,4,5,6)P5 | 0.7 | n.d. | 1.5 | 0.3 | 0.4 | 0.045 | 0.03 | . |
| InsP1 | 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 | . |

1Data are given as LSMeans and pooled SEM (untransformed data); InsP not mentioned here were not detectable. InsP1 isomers were not well separated from co-eluting matrix components and, therefore, not precisely identified. However, concentrations of InsP1 isomers potential including other components were < 1.5 µmol/g DM for all samples.
2Corn–soybean meal-based basal diet.
3Corn–soybean meal-rapeseed cake-based basal diet.
4n.d., not detectable in the majority of samples.
5At least one of the following isomers: Ins(1,2,6)P3, Ins(1,4,5)P3, Ins(2,4,5)P3.
6<LOQ, below limit of quantification in the majority of samples.
7Number of observations for BDbasal and BDbasal without phytase supplementation were n = 5 and n = 4, respectively, and for BDbase and BDbase with phytase supplementation were n = 6, respectively.
8Means in a row not sharing a common superscript differ significantly (multiple t-tests in case of interaction) (P < 0.05).
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BDRSC 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, InsP, and InsP 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 in ileal digesta of pigs fed the BDs were Ins(1,2,3,4,5) and Ins(1,2,4,5,6)P. Their proportions in ΣInsP1-5 were 0.77 and 0.78 for SBM-based BDs for Exp. 1 and Exp. 2, respectively, and 0.64 for BDRSC of Exp. 2. The InsP 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 isomers in ΣInsP 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 ΣInsP isomers in ΣInsP decreased sharply when phytase was supplemented (0.12, 0.09, and 0.08 ΣInsP for supplementation levels 750, 1,500, and 3,000 FTU/kg diet, respectively). Furthermore, the Ins(1,2,5,6)P concentration increased markedly (proportion in ΣInsP: 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 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 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–rapeseed meal (RSM) diet leads to a proportion of ΣInsP in ΣInsP of 0.58, 0.64, and 0.55. This increase in InsP fraction relative to other Ins in ileal digesta of pigs is in accordance with results presented by Kühn et al. (2016).
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.

### 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)$^1$

| Phytozyme | BD$_{SBM}$ | BD$_{RSC}$ | SEM | Protein source | Phytase | Protein source × phytase |
|-----------|------------|------------|-----|---------------|---------|-------------------------|
|           | 0 1,500    | 0 1,500    |     |               |         |                         |
| 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 |
| Try | 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)$^1$

| Phytozyme | BD$_{SBM}$ | BD$_{RSC}$ | SEM | Protein source | Phytase | Protein source × phytase |
|-----------|------------|------------|-----|---------------|---------|-------------------------|
|           | 0 1,500    | 0 1,500    |     |               |         |                         |
| 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$^{ac}$ | 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 $n = 5$.

$^a$Means in a row not sharing a common superscript differ significantly (multiple t-tests in case of interaction) ($P < 0.05$).
especially when phytase was supplemented to the diet. 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 InsP₆ 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 InsP₆ 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 it 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 InsP₆ and digest P of plant origin (Rodehutscord and Rosenfelder, 2016). When phytase was supplemented, more than 90% of InsP₆ was found hydrolyzed in ileal digesta of pigs in Exp. 1 and 85% in Exp. 2. In feces, almost all InsP₆ 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 InsP₆.

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 InsP₆-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 InsP₆ in these 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; Pomer 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 BD₈₅ when compared with BD₉₀ 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
Phytase 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 InsP₆ and the positively charged basic AA residues of Arg, Lys, and His. Furthermore, it is possible that InsP₆ with its multiple binding sites, might be an effective chelator for the cations of metalloenzymes such as carboxylases (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 InsP₆ occurs at pH 3 (Deshpande and Damodaran, 1989) and the pH in the ileum is higher. Furthermore, the amount of “free” InsP₆ 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 (Kaewtapi 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 InsP₆ hydrolysis. However, total tract P digestibility with high level of phytase supplementation hardly exceeded a value of 60%. The Ins(1,2,5,6)P₄ isomer is the main bottleneck of InsP₆ and the digestive enzymes. However, total tract P digestibility with high level of phytase supplementation hardly exceeded a value of 60%. The Ins(1,2,5,6)P₄ isomer is the main bottleneck in the degradation pathway of InsP₆ 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.

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Conflict of interest statement
The authors declare that they have no conflict of interest.

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