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Effects of rapeseed meal fiber content on phosphorus and calcium digestibility in growing pigs fed diets without or with microbial phytase

M. Bournazel[1,2], M. Lessire[1], M. J. Duclos[1], M. Magnin[2], N. Même[1], C. Peyronnet[3], E. Recoules[1], A. Quinsac[4], E. Labussière[5] and A. Narcy[1†]

[1]*URA, INRA, 37380 Nouzilly, France;* [2]*MiXscience, Centre d'affaires Odyssée, ZAC Cicé Blossac, 35172 Bruz, France;* [3]*Terres Univia, 11 rue Monceau, 75008 Paris, France;* [4]*Terres Inovia, 11 rue Monge, Parc Industriel, 33600 Pessac, France;* [5]*PEGASE, Agrocampus Ouest, INRA, Domaine de la Prise, 35590 Saint-Gilles, France*

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The optimization of dietary phosphorus (P) and calcium (Ca) supply requires a better understanding of the effect of dietary fiber content of co-products on the digestive utilization of minerals. This study was designed to evaluate the effects of dietary fiber content from 00-rapeseed meal (RSM) on P and Ca digestibility throughout the gastrointestinal tract in growing pigs fed diets without or with microbial phytase. In total, 48 castrated male pigs (initial BW = 36.1 ± 0.4 kg) were housed in metabolic crates for 29 days. After an 8-day adaptation period, pigs were allocated to one of the eight treatments. The impact of dietary fiber was modulated by adding whole RSM (wRSM), dehulled RSM (dRSM) or dRSM supplemented with 4.5% or 9.0% rapeseed hulls (dRSMh1 and dRSMh2). Diets contained 0 or 500 phytase unit of microbial phytase per kg. From day 14 to day 23, feces and urine were collected separately to determine apparent total tract digestibility (ATTD) and apparent retention (AR) of P and Ca. At the end of the experiment, femurs and digestive contents were sampled. No effect of variables of interest was observed on growth performance. Microbial phytase increased ATTD and AR of P ($P < 0.001$*) but the P equivalency with the wRSM diet was lower than expected. Moreover, stomach inorganic P (iP) solubility was improved by microbial phytase (*$P < 0.001$*). The ATTD of Ca was not affected by microbial phytase which increased AR of Ca and femur characteristics (*$P < 0.05$*). Ileal recovery of P was not affected by microbial phytase but cecal recovery was considerably reduced by microbial phytase (*$P < 0.001$*). The decrease in digesta pH between the distal ileum and cecum (7.6 v. 5.9) enhanced the solubility of iP and may have improved its absorption, as supported by the negative relationship between soluble iP and pH (*$R^2 = 0.40$*, $P < 0.001$ without microbial phytase and $R^2 = 0.24$, $P = 0.026$ with microbial phytase). The inclusion of hulls improved the solubility of iP (*$P < 0.05$*). In conclusion, dehulling does not largely increase nutrient digestibility although dRSM seems to improve the efficacy of microbial phytase in releasing phosphate in the stomach. Moreover, dietary fiber may affect solubilization process in the cecum which potentiates the effect of microbial phytase on P digestibility.*

Keywords: calcium, dietary fiber, microbial phytase, phosphorus, rapeseed meal

Implications

Dehulling is a relevant process to reduce the fiber content of rapeseed meal and to concentrate protein and energy fraction. However it seems to have a low impact on phosphorus (P) and calcium (Ca) digestibility, whereas it improves phosphorus equivalency of microbial phytase. This implies that phosphorus equivalencies need to be adapted according to the type of rapeseed meal used and to the fiber level.

† E-mail: agnes.narcy@inra.fr

Introduction

The inclusion of co-products, such as 00-rapeseed meal (RSM), in pig diets increased over the last decade because of its higher availability and its low glucosinolates and erucic acid content. Nevertheless, RSM is characterized by a relatively high dietary fiber and phytate content (0.50%) which are considered as anti-nutritional factors. Procedures, such as dehulling the seed, are of great interest to reduce the dietary fiber fraction which is mainly located in the hulls (Carré *et al.*, 2015). However, the impact of dietary fiber of RSM on P digestibility remains unknown whereas dietary

provision of P for pigs is still an environmental and economic challenge. Previous studies focused on measuring the apparent, true or standardized digestibility of P in diets with RSM, but did not investigate the specific effect of RSM fiber on digestive process (Adhikari *et al*., 2015; Shi *et al*., 2015).

According to the literature, the effects of dietary fiber on P digestibility in pigs are not consistent and depend strongly on the source and the inclusion level (Metzler and Monsenthin, 2008). Dietary fiber can affect digestive process in the upper gastrointestinal tract because of their physico-chemical properties (Jha and Berrosco, 2015), and consequently phytate degradation by microbial phytase (Kemme *et al*., 2006; Dersjant-Li *et al*., 2015). The decrease of the pH between the terminal ileum and the cecum (from 7.0 to 6.0) due to microbial breakdown of fiber may improve the solubility of minerals, thereby increasing their absorption (Scholz-Arhens and Schrezenmeir, 2007). In addition, dietary fiber could affect Ca digestibility (Nortey *et al*., 2008) modifying in this way Ca and P balance and consequently bone mineralization. In this regard, we hypothesized that (1) dehulling the seed could improve nutrient digestibility by reducing the fiber matrix effect, (2) the hull content could lead to fermentations in the lower intestine enhancing mineral solubility and (3) the response to microbial phytase might be affected by RSM inclusion.

Therefore, the aim of the present study was to investigate the effects of dietary fiber content of RSM on P and Ca digestibility as well as on bone mineralization in growing pigs fed diets without or with microbial phytase, and to evaluate their impacts on P and Ca solubility in relation to the pH, and their recovery throughout the gastrointestinal tract.

Material and methods

The experimental protocol was approved by the Regional Ethics Committee on animal experimentation (Rennes, France) and the French Ministry of Higher Education and Research (Paris, France; authorization: 02402.03).

Raw materials and experimental diets

The RSM used was a 00-rapeseed meal. From a single batch of seed, whole RSM and dehulled RSM were processed at the CREOL pilot plant (Pessac, France; Supplementary Table S1). The dehulling of seed was performed before oil extraction and hulls were collected. Extraction was done using hexane.

The eight experimental diets were based on 67% corn and 10% soybean meal. Phosphorus and Ca were marginally deficient in diets without microbial phytase according to the French recommendations for 40 kg pigs (Jondreville and Dourmad, 2005). The dietary fiber content of the diets was modulated by adding RSM. The first diet (wRSM) included 15% whole RSM to adjust the protein concentration to 15% (Table 1). The second diet (dRSM) included 10.5% dehulled RSM to provide similar amounts of protein to wRSM. The third and fourth diets (dRSMh1 and dRSMh2, respectively) included 10.5% dRSM and 4.5% or 9.0% hulls, respectively. Diets were supplemented with 0 or 500 phytase units (FTU) microbial phytase per kg (Natuphos® from *Aspergillus niger*, BASF SE, Ludwigshafen, Germany). The level of apparent digestible P (Sauvant *et al*., 2004) was fixed at 0.16% in diets without microbial phytase and at 0.24% in diets supplemented with microbial phytase, in accordance with the French recommendations, considering an equivalency of 0.8 g/kg of apparent digestible P with 500 FTU microbial phytase/kg (Jondreville and Dourmad, 2005). The Ca : apparent digestible P ratio was adjusted to 2.6 using calcium carbonate. Titanium dioxide (TiO_2, 0.30%) was added as an indigestible marker. The diets were offered to pigs as pellets.

Animals and experimental procedures

The experiment included 48 castrated male Piétrain × (Landrace × Large White) pigs weighing 36.1 ± 0.4 kg. Animals were housed in crates equipped with a slatted floor with a tray underneath that allowed separation of urines and feces collection. From day 0 to day 7, the pigs received a standard diet covering all their nutrient requirements (9.7 MJ net energy and 18% CP). At day 8, pigs were assigned to one of the eight experimental diets (six pigs/diet) in a complete randomized block design (six blocks of eight treatments). The pigs were offered an equal quantity of feed daily distributed in two equivalent meals corresponding to 4% of their BW. Daily feed intake was recorded individually during the experimental period. Collection period started at day 14 and ended at day 23 after an overnight fast to ensure the emptying of the intestine. Feces were collected at meal times (twice a day) and pooled by pig. At the end of the collection period, feces were mixed thoroughly with water, sampled, weighed and freeze-dried. Acidified urine (1% of the total, with 3 ml of 10% sulfuric acid/l of urine) was also collected individually each day and stored at −20°C. Pigs were weighed at day 0, day 8, day 14 and day 23. Room temperature was maintained at 20 ± 1°C. At the end of the experiment, individual pigs received half of their daily feed allowance in the morning and the other half 2 h 30 min before slaughter. They were anesthetized by electro-immobilization and slaughtered by exsanguination. The viscera were immediately removed and samples of digesta were collected from stomach, duodenum, jejunum (from the end of duodenum to the beginning of ileum), proximal ileum (1 m before distal ileum), distal ileum (80 cm anterior to the ileocecal valve), cecum and middle of the colon, homogenized and stored at −80°C. A sample of digesta from the cecum and the distal ileum was freeze-dried for 48 h and then ground. Femurs were also collected and stored at −20°C.

Analyses

All samples were analyzed in duplicate. The crude fiber content was measured with the Weende method (NF V03 040), and NDF, ADF and ADL were obtained according to Van Soest *et al.* (1991). Soluble and insoluble fibers were determined according to Prosky method (AOAC 991.42 and 993.19). The initial pH and the buffering capacity to pH 3 were measured according to Lawlor *et al.* (2005). Dry matter of diets, feces and digestive content of ileum and cecum, and P, Ca and TiO_2

analysis were performed as previously explained by Rousseau *et al.* (2016). Phytase activity was measured according to Engelen *et al.* (1994). Levels of Ca and P content were measured in diets, feces and urine to determine apparent total tract digestibility (ATTD) and apparent retention (AR). Levels of P, Ca and TiO_2 were measured in diets and digesta to determine Ca and P recovery. The soluble Ca and inorganic P (*i*P) levels were determined in the stomach and cecal supernatant fractions, after centrifuging the samples at 23 000 **g** for 10 min at 4°C. Inorganic P was analyzed using a kit (Kit Phosphore UV 61571; bioMérieux, 69280 Marcy l'Etoile, France). Nitrogen (NF EN ISO 5983-2) and fat (NF ISO 6492) were determined in diets. The pH was measured on all digestive contents with a pH meter (InLab Solid Pro-ISM with SevenGo Duo™ SG23; Mettler Toledo, Greifensee, Switzerland). Right femurs were broken and then defatted using ether, dried at 103°C for 18 h and finally ashed in a muffle furnace at 600°C for 16 h. The left femurs were analyzed using computed tomography (Siemens Emotion Duo, Erlangen, Germany). Bone densitometry (BD) was determined using X-ray absorbance: mineral density was proportional to grayscale value in Hounsfield units (HU), from 250 to 1500 HU.

Calculations and statistical analysis

Ileal and cecal recovery were calculated using the following equation:

$$\text{Recovery} = \left(X_{\text{diet}}/X_{\text{digesta}}\right) \times \left(\text{TiO}_{2\text{digesta}}/\text{TiO}_{2\text{diet}}\right)$$

Where X is the amount of P or Ca in the diet or in the digesta (mg/g DM) and TiO_2 the amount of TiO_2 in the diet or in the digesta (mg/g DM).

The P equivalency values were calculated using the ATTD of P and the dietary P content following the method of Kerr *et al.* (2010), and the analyzed values of phytase activity.

All data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA) as appropriate for a randomized complete block design. Crates location was the blocking factor and pigs were the experimental unit. The model included diets as fixed effect and block as random effect. Orthogonal polynomial contrasts were used to separate treatment means. Contrasts were chosen to study the effects of (1) microbial phytase (linear (Lin), mean of diets without phytase *v.* mean of diets with phytase), (2) dehulled rapeseed without microbial phytase (Lin, wRSM− *v.* dRSM−) and (3) with microbial phytase (Lin, wRSM+ *v.* dRSM+), (4) the hull content without microbial phytase (Lin, quadratic (Qua), dRSM− *v.* dRSMh1− *v.* dRSMh2−) and (5) with microbial phytase (Lin, Qua, dRSM+ *v.* dRSMh1+ *v.* dRSMh2+). Differences were considered significant at $P < 0.05$ and to have a tendency at $0.05 \leqslant P < 0.10$. Relationships between the pH and the mineral solubility in the stomach and in the cecum were quantified using regression analysis with the REG procedure of SAS. The relation between the *i*P solubility in the stomach and BD was estimated using a quadratic regression model as well as broken-line and curvilinear-plateau regression models. Estimates were obtained using REG and NLIN procedures of SAS (Supplementary Figure S1).

Results

Nutritional values of experimental diets

The P and Ca concentrations analyzed in the experimental diets were in accordance with the expected values (Table 1). Analyzed phytase activity was 650, 450, 870 and 630 FTU/kg in wRSM+, dRSM+, dRSMh1+ and dRSMh2+ diets, respectively, and less than the detection limit of 100 FTU/kg in the diets without microbial phytase. As expected, NDF, ADF, ADL, insoluble and soluble fiber contents decreased when including dRSM (7.8%, 2.9%, <0.5%, 1.2% and 10.7%, respectively). The dRSMh1 diet contained almost the same level of fibers as wRSM while dRSMh2 presented the highest level of fibers (13.1%, 7.3%, 2.8%, 1.8% and 15.5%, respectively).

Growth performance and bone mineralization

There was no significant effect of microbial phytase, dehulled seed and hull content on BW at day 8, day 23 or on average daily gain between day 8 and day 23 (Table 2). The inclusion of hulls without microbial phytase influenced the feed conversion ratio (Qua; $P = 0.037$) and the inclusion of hulls with microbial phytase increased the average daily feed intake ($P = 0.017$). Microbial phytase increased femur ash weight ($P < 0.001$) and content ($P = 0.005$), and bone density ($P < 0.001$).

Phosphorus and calcium balance

Microbial phytase decreased P fecal excretion (−25%, $P < 0.001$; Table 3), increased P urinary excretion (+30%, $P = 0.020$), ATTD of P (+18.4 points; $P < 0.001$), AR of P (+17.6 points; $P < 0.001$), Ca fecal excretion (+33%, $P < 0.001$) and AR of Ca (+6.7 points; $P = 0.011$). With microbial phytase, the inclusion of hulls affected P urinary excretion (+36%; Qua, $P = 0.040$). The P equivalency values were 0.48, 0.69, 0.88 and 0.84 g of apparent digestible P/kg of diet for wRSM, dRSM, dRSMh1 and dRSMh2, respectively.

pH of digestive contents and stomach solubility, ileal recovery and cecal solubility and recovery of phosphorus and calcium

Microbial phytase increased duodenum ($P = 0.031$) and colon pH ($P = 0.017$; Table 4) except in the duodenum of pigs fed the wRSM diet. The dRSM− diet increased jejunum ($P = 0.006$) and cecum pH ($P < 0.001$) compared with wRSM−. Without microbial phytase, the inclusion of hulls decreased cecum pH (Lin, $P = 0.006$). Total and soluble P and Ca in the stomach and cecum are presented in the Supplementary Table S2. Microbial phytase increased *i*P solubility in the stomach ($P < 0.001$; Table 4) and decreased *i*P solubility ($P = 0.008$) and P recovery in the cecum ($P < 0.001$). Without microbial phytase, the inclusion of hulls increased *i*P solubility (Lin and Qua, $P < 0.001$) and recovery of Ca in the cecum (Lin, $P = 0.017$ and Qua, $P = 0.033$). With microbial phytase, the inclusion of hulls increased *i*P solubility (Lin, $P = 0.026$ and Qua, $P = 0.014$) and decreased Ca solubility (Lin, $P = 0.001$ and Qua, $P = 0.032$).

Table 1 *Ingredients and chemical composition of experimental diets (as-fed basis)*

	wRSM		dRSM		dRSMh1		dRSMh2	
	−	+	−	+	−	+	−	+
Ingredients (%)								
Corn	67.01	67.01	67.00	67.00	66.99	66.99	66.99	66.99
Soybean meal	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Rapeseed meal	15.00	15.00	–	–	–	–	–	–
Dehulled rapeseed meal	–	–	10.50	10.50	10.50	10.50	10.50	10.50
Rapeseed hulls	–	–	–	–	4.50	4.50	9.00	9.00
Cornstarch	5.78	5.24	9.99	9.45	5.73	5.28	1.43	0.90
Calcium carbonate	0.45	0.99	0.56	1.10	0.44	0.98	0.33	0.86
Dicalcium phosphate	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Sodium chloride	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Titanium dioxide	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Sodium bicarbonate	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
L-Lysine HCl	0.36	0.36	0.44	0.44	0.40	0.36	0.36	0.36
DL-Methionine	0.03	0.03	0.06	0.06	0.05	0.03	0.03	0.03
L-Threonine	0.09	0.09	0.13	0.13	0.10	0.08	0.08	0.08
L-Tryptophan	0.03	0.03	0.04	0.04	0.04	0.03	0.03	0.03
L-Valine	–	–	0.03	0.03	–	–	–	–
Nutritional characteristics[1] (%)								
DM	87.3	88.5	88.0	87.9	87.3	88.2	87.4	88.7
Phytase (FTU/kg)	<100	650	<100	450	<100	870	<100	630
Ca	0.44	0.62	0.40	0.58	0.44	0.58	0.42	0.60
Total P	0.47	0.44	0.44	0.43	0.45	0.46	0.46	0.47
digP[2,3]	0.16	0.24	0.16	0.24	0.16	0.24	0.16	0.24
Ca : digP	2.8	2.6	2.6	2.5	2.8	2.4	2.7	2.5
Fat	3.0	3.1	2.9	2.7	2.9	2.8	3.2	3.0
CP (N × 6.25)	15.4	14.6	14.6	14.5	14.7	14.3	15.2	15.3
NDF	10.5	10.5	7.8	7.8	10.0	10.0	13.1	13.1
ADF	5.1	5.1	2.9	2.9	5.3	5.3	7.3	7.3
ADL	1.6	1.6	<0.5	<0.5	1.6	1.6	2.8	2.8
Soluble fiber	1.7	1.7	1.2	1.2	1.3	1.3	1.8	1.8
Insoluble fiber	13.1	13.1	10.7	10.7	12.7	12.7	15.5	15.5
Net energy (MJ/kg[2])	9.95	9.89	10.30	10.24	9.86	9.80	9.41	9.36
Initial pH	6.9	7.0	7.1	7.0	7.0	6.9	6.9	6.9
BUF 3	147	158	123	153	124	165	122	162

wRSM = whole rapeseed meal; dRSM = dehulled rapeseed meal; dRSMh1 = dehulled rapeseed meal with 4.5% rapeseed hulls; dRSMh2 = dehulled rapeseed meal with 9% rapeseed hulls (− = without microbial phytase and + = with 500 FTU/kg); BUF = buffering capacity to pH 3.
[1]Analyzed values (as described in 'Material and methods' section).
[2]Calculated values.
[3]Apparent digestible phosphorus (Sauvant *et al.*, 2004).

Regression analysis

With microbial phytase, soluble *i*P was negatively correlated with the pH of the stomach content ($R^2 = 0.31$, $P = 0.009$; Figure 1a) but no effect was observed without microbial phytase. Soluble Ca was negatively correlated with the pH of the stomach content without microbial phytase ($R^2 = 0.53$, $P < 0.001$; Figure 1b) and with MP ($R^2 = 0.85$, $P < 0.001$). Soluble *i*P in the cecum was negatively correlated with pH without ($R^2 = 0.40$, $P < 0.001$; Figure 2) and with microbial phytase ($R^2 = 0.24$, $P = 0.026$).

Discussion

Using dRSM instead of wRSM or adding hulls did not affect the ATTD or AR of P and Ca. However, the P equivalency value was twofold lower with the wRSM diet than expected (0.48 *v.* 0.83 g/kg; Kerr *et al.*, 2010). Moreover, the solubility of *i*P in the stomach tended to be enhanced with dehulled seeds in diets supplemented with microbial phytase. These results suggest that dehulling the seed enhances the action of microbial phytase which takes place mainly in the stomach. Phytate is distributed throughout the kernel in sub-cellular inclusions known as globoids (Singh, 2008). Removing hulls from the seed may reduce binding and physical entrapment of phytate, improving in this way the accessibility to the enzyme (Baye *et al.*, 2015). This hypothesis is confirmed by the fact that the positive effect is remaining with the dRSMh1 + diet although it almost contains the same level of fibers as wRSM + . In addition, neither dehulled seeds nor the inclusion of hulls significantly affected

Table 2 *Growth performance and femurs characteristics in pigs fed experimental diets namely whole rapeseed meal (wRSM), dehulled RSM (dRSM) or dRSM supplemented with 4.5% or 9.0% rapeseed hulls (dRSMh1 and dRSMh2, respectively)*[1]

										P-value[2]						
Description	wRSM		dRSM		dRSMh1		dRSMh2			Effect of microbial phytase	Effect of dRSM		Effect of hulls content			
MP suppl.[3]	−	+	−	+	−	+	−	+		1, 3, 5, 7 *v.* 2, 4, 6, 8	1 *v.* 3	2 *v.* 4	3 *v.* 5 *v.* 7		4 *v.* 6 *v.* 8	
Diets	1	2	3	4	5	6	7	8	SEM	Lin	Lin	Lin	Lin	Qua	Lin	Qua
BW day 8 (kg)	40.3	40.4	40.1	39.8	39.5	40.7	40.2	40.4	0.3	0.54	0.81	0.57	0.94	0.67	0.57	0.85
BW day 23 (kg)	52.8	53.2	52.6	52.3	52.0	53.4	52.0	52.8	0.3	0.28	0.80	0.36	0.56	0.74	0.62	0.92
ADG (g/day)	833	850	833	828	833	850	789	822	15	0.36	1.00	0.50	0.19	0.13	0.87	0.56
ADFI (kg/day)	1.58	1.58	1.59	1.56	1.58	1.58	1.57	1.60	0.01	0.51	0.56	0.093	0.22	0.30	0.017	0.36
FCR	1.87	1.88	1.93	1.89	1.85	1.88	2.02	1.97	0.03	0.76	0.39	0.86	0.20	0.037	0.31	0.20
Femurs characteristics																
Dry weight (g)	83.8	91.4	89.0	85.2	81.4	97.0	87.1	88.8	1.5	0.063	0.36	0.27	0.73	0.70	0.51	0.63
Ash weight (g)	31.6	36.8	32.3	36.0	29.9	38.0	32.3	36.7	0.6	<0.001	0.63	0.55	1.00	0.31	0.62	0.79
Ash (% DM)	38.3	40.3	36.5	42.6	37.0	39.4	37.6	41.3	0.6	0.005	0.45	0.36	0.63	0.67	0.61	0.86
BD (cm^2)	6.40	8.44	6.93	7.75	5.99	8.50	6.91	8.27	0.17	<0.001	0.19	0.090	0.97	0.19	0.19	0.66

ADG = average daily gain; ADFI = average daily feed intake; FCR = feed conversion ratio; BD = bone density: area greater than 250 HU; Lin = linear; Qua = quadratic.
[1]*n* = 6 pigs per diet.
[2]Contrast analysis: linear effect of microbial phytase; linear effect of dehulled rapeseed seed without and with microbial phytase; linear and quadratic effects of the inclusion of hulls without and with microbial phytase.
[3]Microbial phytase supplementation: − = without microbial phytase and + = with 500 FTU/kg.

Table 3 *Intake, fecal excretion, urinary excretion, apparent total tract digestibility (ATTD), apparent retention (AR) in pigs fed experimental diets namely whole rapeseed meal (wRSM), dehulled RSM (dRSM) or dRSM supplemented with 4.5% or 9.0% rapeseed hulls (dRSMh1 and dRSMh2, respectively)*[1]

										P-value[2]						
Description	wRSM		dRSM		dRSMh1		dRSMh2			Effect of microbial phytase	Effect of dRSM		Effect of hulls content			
MP suppl.[3]	−	+	−	+	−	+	−	+		1, 3, 5, 7 *v.* 2, 4, 6, 8	1 *v.* 3	2 *v.* 4	3 *v.* 5 *v.* 7		4 *v.* 6 *v.* 8	
Diets	1	2	3	4	5	6	7	8	SEM	Lin	Lin	Lin	Lin	Qua	Lin	Qua
Phosphorus																
Intake (g/day)	7.10	6.84	6.70	6.58	6.92	6.98	6.99	7.19	0.03	0.011	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Fecal excretion (g/day)	5.01	3.90	4.94	3.62	5.25	3.78	5.26	3.99	0.11	<0.001	0.76	0.19	0.14	0.37	0.087	0.12
Urinary excretion (g/day)	0.09	0.13	0.11	0.11	0.10	0.11	0.10	0.15	0.01	0.020	0.25	0.56	0.78	0.97	0.069	0.040
ATTD (% of intake)	29.5	43.0	26.3	45.0	24.1	45.8	24.7	44.5	1.6	<0.001	0.29	0.51	0.60	0.85	0.87	0.73
AR (% of intake)	28.2	39.8	24.5	43.2	22.5	43.8	23.0	42.0	1.5	<0.001	0.25	0.29	0.65	0.87	0.70	0.58
Calcium																
Intake (g/day)	6.68	9.49	6.18	8.81	6.66	8.83	6.77	9.27	0.19	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Fecal excretion (g/day)	3.61	4.64	3.07	4.14	3.46	4.16	3.33	4.83	0.13	<0.001	0.18	0.21	0.52	0.85	0.089	0.055
Urinary excretion (g/day)	0.81	0.52	0.68	0.71	0.59	0.54	0.56	0.45	0.04	0.15	0.40	0.18	0.36	0.50	0.059	0.14
ATTD (% of intake)	50.3	51.2	50.4	53.1	48.1	52.9	50.9	50.6	0.9	0.28	0.98	0.59	0.89	0.59	0.50	0.48
AR (% of intake)	32.6	45.7	39.3	45.0	39.3	46.8	42.7	43.2	1.4	0.011	0.19	0.90	0.51	0.44	0.72	0.54

Lin = linear; Qua = quadratic.
[1]*n* = 6 pigs per diet.
[2]Contrast analysis: linear effect of microbial phytase; linear effect of dehulled rapeseed seed without and with microbial phytase; linear and quadratic effects of the inclusion of hulls without and with microbial phytase.
[3]Microbial phytase supplementation: − = without microbial phytase and + = with 500 FTU/kg.

Table 4 *Phosphorus and calcium solubility in the stomach and the cecum, recovery in the ileum and cecum and pH of digesta in different segments of the digestive tract in pigs fed experimental diets namely whole rapeseed meal (wRSM), dehulled RSM (dRSM) or dRSM supplemented with 4.5% or 9.0% rapeseed hulls (dRSMh1 and dRSMh2, respectively, DM basis)*[1]

										P-value[2]						
Description	wRSM		dRSM		dRSMh1		dRSMh2			Effect of microbial phytase	Effect of dRSM		Effect of hulls content			
MP suppl.[3]	−	+	−	+	−	+	−	+		1, 3, 5, 7 *v.* 2, 4, 6, 8	1 *v.* 3	2 *v.* 4	3 *v.* 5 *v.* 7		4 *v.* 6 *v.* 8	
Diets	1	2	3	4	5	6	7	8	SEM	Lin	Lin	Lin	Lin	Qua	Lin	Qua
pH of digestive content																
Stomach	5.26	5.53	5.55	5.56	5.45	5.45	5.18	5.64	0.05	0.096	0.17	0.88	0.087	0.090	0.72	0.47
Duodenum	5.79	5.69	5.77	6.02	5.61	6.09	5.48	6.03	0.07	0.031	0.94	0.20	0.22	0.31	0.94	0.92
Jejunum	6.36	6.45	6.59	6.44	6.50	6.49	6.46	6.49	0.02	0.73	0.006	0.85	0.10	0.21	0.55	0.74
Proximal ileum	7.36	7.49	7.53	7.20	7.65	7.44	7.41	7.28	0.05	0.19	0.41	0.16	0.58	0.33	0.69	0.81
Distal ileum	7.52	7.79	7.57	7.44	7.63	7.68	7.60	7.50	0.05	0.80	0.77	0.062	0.89	0.99	0.72	0.73
Cecum	5.75	6.05	6.14	5.93	6.03	5.97	5.84	5.85	0.03	0.84	<0.001	0.28	0.006	0.010	0.45	0.29
Colon	6.06	6.36	6.03	6.28	6.07	6.12	6.01	6.23	0.05	0.017	0.87	0.63	0.91	0.78	0.73	0.88
Inorganic phosphorus solubility (%)																
Stomach	9.97	17.50	8.43	21.92	9.51	20.60	9.74	18.11	0.93	<0.001	0.49	0.067	0.56	0.69	0.11	0.12
Cecum	2.55	1.87	1.97	1.92	2.55	1.99	3.36	2.71	0.11	0.008	0.12	0.88	<0.001	<0.001	0.026	0.014
Calcium solubility (%)																
Stomach	6.84	8.99	5.78	7.95	7.59	9.37	8.03	6.54	0.44	0.20	0.55	0.56	0.21	0.40	0.40	0.16
Cecum	1.17	1.30	0.98	1.61	1.05	0.99	1.20	0.93	0.06	0.25	0.35	0.12	0.29	0.30	0.001	0.032
Phosphorus recovery																
Ileum	0.55	0.54	0.59	0.49	0.58	0.51	0.62	0.60	0.02	0.13	0.50	0.40	0.66	0.52	0.087	0.083
Cecum	0.50	0.25	0.52	0.29	0.49	0.34	0.57	0.33	0.02	<0.001	0.68	0.48	0.31	0.14	0.45	0.77
Calcium recovery																
Ileum	0.56	0.64	0.61	0.59	0.61	0.66	0.54	0.68	0.02	0.094	0.57	0.50	0.41	0.34	0.25	0.43
Cecum	0.59	0.54	0.52	0.53	0.57	0.56	0.62	0.60	0.01	0.33	0.094	0.84	0.017	0.033	0.082	0.11

Lin = linear; Qua = quadratic.
[1]$n = 6$ pigs per diet.
[2]Contrast analysis: linear effect of microbial phytase; linear effect of dehulled rapeseed seed without and with microbial phytase; linear and quadratic effects of the inclusion of hulls without and with microbial phytase.
[3]Microbial phytase supplementation: − = without microbial phytase and + = with 500 FTU/kg.

the absorption of P and Ca in the small intestine. There is some evidence that fiber can modulate the absorption of minerals from the upper gastrointestinal tract (Metzler and Monsenthin, 2008). The estimated content in hemicelluloses fractions was increased by almost 20% between dRSM and wRSM or dRSMh2 diets. The ability of hemicelluloses to bind Ca is particularly high at neutral pH, especially compared with lignin (Claye *et al.*, 1998). The level of inclusion of hulls in the present experiment might have been too low to induce significant effects in the small intestine on mineral availability. The hull content of the diet did not affect the cecal recovery of P. Nevertheless, increasing the level of hulls reduced the pH of the cecal content (6.14 *v.* 5.84) in pigs fed diets without microbial phytase and slightly improved the solubility of *i*P in the cecal content. Fiber from hulls, although mostly insoluble, may have been fermented and thus lowered the pH. The higher acidity produced through fermentation resulted in greater solubility and availability of P. Indeed, both soluble and insoluble dietary fiber can be degraded by intestinal bacteria, but insoluble fiber is less easily, rapidly and completely fermented than soluble fiber (Bach Knudsen and Hansen, 1991). The effects of fiber on mineral absorption in the hindgut are controversial. The source and the inclusion level of dietary fiber have a considerable effect on P absorption or secretion in the large intestine as a result of the different bacteria P needs for fermentation (Metzler and Monsenthin, 2008). For instance, an increase in the cellulose content of the diet from 3% to 9% was reported to negatively affect the absorption of P from the large intestine (Partridge, 1978b). In contrast, some studies using various types of fiber have suggested greater P absorption (Den Hartog *et al.*, 1988; Heijnen and Beynen, 1998). The cecal recovery of Ca was higher when the inclusion level of hulls was increased (from 0.53 to 0.61 on average for dRSM and dRSMh2, respectively). Moreover, although low, the solubility of Ca was reduced when the hull

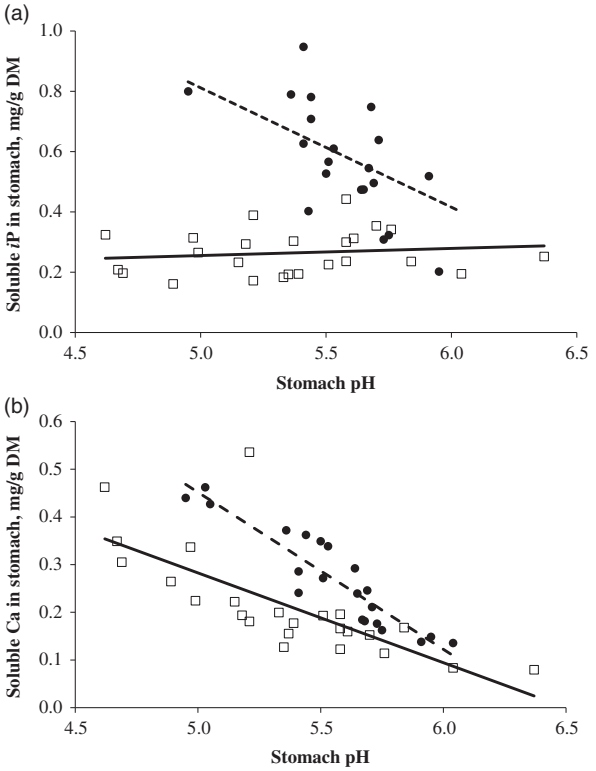

Figure 1 Relation between stomach pH and stomach solubility of P (a) and Ca (b) in pigs fed diets without microbial phytase (□) or with microbial phytase (●). (a) Without microbial phytase: $y = 0.139 + 0.027x$, $R^2 = 0.02$, $P = 0.52$, $n = 24$; with microbial phytase: $y = 2.790 - 0.396x$, $R^2 = 0.31$, $P = 0.009$, $n = 20$. (b) Without microbial phytase: $y = 1.223 - 0.188x$, $R^2 = 0.53$, $P < 0.001$, $n = 24$; with microbial phytase: $y = 2.098 - 0.329x$, $R^2 = 0.85$, $P < 0.001$, $n = 21$.

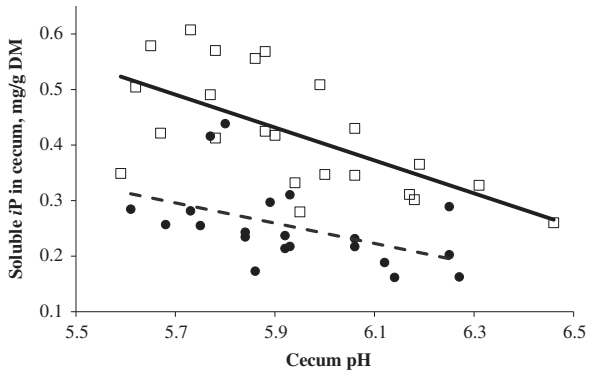

Figure 2 Relation between cecum pH and cecum solubility of P in pigs fed diets without microbial phytase (□) and with microbial phytase (●). Without microbial phytase: $y = 2.178 - 0.296x$, $R^2 = 0.40$, $P < 0.001$, $n = 24$; with microbial phytase: $y = 1.337 - 0.183x$, $R^2 = 0.24$, $P = 0.026$, $n = 21$.

content in diets supplemented with microbial phytase was increased. These results can be explained by the higher Ca requirement to buffer the excess of acidity in the cecum with progressing fermentation as illustrated by Metzler-Zebeli *et al.* (2010) who reported a negative relationship between volatile fatty acids in feces and net post-ileal Ca absorption.

Microbial phytase significantly improved the ATTD of P from 26.1% to 44.6%. A similar improvement in the ATTD of P from 28% to 56% was previously reported when phytase was added (863 FTU/kg) to a diet containing 13.2% canola meal (Akinmusire and Adeola, 2009). Microbial phytase supplementation led to an increase of Ca intake as dietary Ca level was adjusted using calcium carbonate in order to keep an equal Ca : apparent digestible P ratio. However, microbial phytase had no impact on ATTD of Ca. This result is particularly surprising since microbial phytase is well known to improve Ca digestibility in canola meal as shown by González-Vega *et al.* (2013). However, in our experiment, calcium carbonate was added and its level was twice as high in diets supplemented with microbial phytase. According to González-Vega *et al.* (2015a), Ca from calcium carbonate is more easily bound to phytate, less soluble or less digestible than other sources which could explain the lack of significant effect of microbial phytase on ATTD of Ca. Calcium AR was significantly improved in pigs fed diets supplemented with microbial phytase (45.2% *v.* 38.5%) with a reduction in urinary Ca (from 21% to 12% of the absorbed Ca). Microbial phytase also caused a large improvement of bone ash content, as previously reported in finishing pigs fed diets supplemented with 1000 FTU of microbial phytase/kg which led to an 8 percentage units increase compared with unsupplemented diets (Varley *et al.*, 2010). These observations are related to the greater availability of P for bone mineralization and illustrate the close relationship between P and Ca at the metabolic level. Both minerals are deposited together in the skeleton to form hydroxyapatite crystals and the element in excess compared with the other is excreted in the urine (Létourneau-Montminy *et al.*, 2010).

There was a strong negative relationship between stomach pH and *i*P solubility in pigs fed diets supplemented with microbial phytase. The presence of feed in the stomach induces distension, with a subsequent increase in intragastric pressure that stimulates acid secretion (Yen, 2001). The relatively acidic pH (5.45) is particularly favorable to phytate solubility and phytase activity (Yi and Kornegay, 1996). In consequence, a large amount of phosphate was released in this compartment. There was also a negative relationship between stomach pH and Ca solubility in pigs fed diets without or with microbial phytase. Maintenance of a low stomach pH may thus prevent the formation of Ca-phosphate complexes. Moreover, the higher level of provision of calcium carbonate in the diets supplemented with microbial phytase led to a higher buffering capacity of the feed (129 *v.* 159). This caused a slight increase in pH of the stomach content (5.36 *v.* 5.54) which remained sufficiently low for optimal activity of microbial phytase which is between pH 2.5 and 5.5 (Dersjant-Li *et al.*, 2015). Solubilization and hydrolysis processes occurring in the stomach proved to be limiting steps in P utilization (Kemme *et al.*, 2006). This was supported by the positive relationship between *i*P solubility in the stomach and bone density as the skeleton is the main storage site of P in the body (c.f. Supplementary Material). However, it is worthy to note that pH

values in the stomach are higher than those generally found in the literature and must be taken with care. The δ between sampling and pH measurement may explain these results as a consequence of acid secretion disruption. Microbial phytase did not have any effect on the ileal recovery of P while a significant decrease in cecal recovery occurred. These results suggest that part of the absorption of P occurred in the cecum, which potentiates the effects of microbial phytase through the absorption of P released by enzymes in the proximal part of the digestive tract. The small intestine is the major site of P absorption (Breves and Schröder, 1991). The role of the large intestine in the regulation of P absorption is more controversial. Some authors have reported secretion of P in the large intestine (Partridge, 1978a; Partridge *et al*., 1986), whereas others have reported significant absorption in both the cecum and the proximal colon (Liu *et al*., 2000). A significant decrease in the pH of the digestive content from the ileum to the cecum occurred (from 7.6 to 5.9), presumably due to volatile fatty acids production resulting from fiber fermentation. Results of previous studies showed a decrease in pH of 6.6 to 7.2 near the ileocecal junction to 5.7 to 6.8 in the cecum of pigs fed various wheat- or oat-based diets (Bach Knudsen and Hansen, 1991). Moreover, a strong negative relationship between the pH of the digesta of the cecum and the solubility of *i*P was found. The low pH in the large intestine might have resulted in the improvement of the solubility of Ca and phosphate, with a subsequent increase in their absorption (González-Vega *et al*., 2015b). It is of note that the solubility of both P and Ca in the cecum was extremely low (around 2.4%). This could be attributed to the buffering functions of Ca and phosphate in order to compensate for the low pH. Indeed, Ca and phosphate have a major role for the buffering capacity throughout the intestinal lumen, forming insoluble complexes at pH values above 6 (Lawlor *et al*., 2005; Selle *et al*., 2009). The higher Ca intake in pigs fed diets with phytase caused an increase in the flow of Ca entering the large intestine, which might explain the higher pH of the digesta of the colon observed with microbial phytase.

In conclusion, the present findings indicate that dietary fiber content of RSM does not have a strong effect on digestive processes in the proximal part of the gastrointestinal tract of pigs. However, dehulling of RSM improved the availability of phytate and the phosphate released in the stomach. This study showed that the large intestine has a significant role in the absorption of P and potentiates the effect of microbial phytase due to its low pH. Dietary fiber from rapeseed induces acidification of the cecal content, thereby improving *i*P solubility, which may improve its absorption.

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Supplementary material

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