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Ileal microbiota of growing pigs fed different dietary calcium phosphate levels and phytase content and subjected to ileal pectin infusion[1]

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ABSTRACT: Two experiments with growing pigs were conducted to determine the effects of dietary P and Ca levels, phytase supplementation, and ileal pectin infusion on changes in bacterial populations in the ileum and on ileal and fecal fermentation patterns. Growing pigs (BW 30.1 ± 1.3 kg) were fitted with simple T-cannulas at the distal ileum and were fed a low-P corn-soybean meal control diet (3 g of P/kg), or the control diet supplemented with either 15 g of monocalcium phosphate (MCP)/kg (Exp. 1) or 1,000 phytase units of phytase/kg (Exp. 2). Daily infusion treatments consisted of either 60 g of pectin dissolved in 1.8 L of demineralized water or 1.8 L of demineralized water as a control infusion, infused via the ileal cannula. In each experiment, 8 barrows were assigned to 4 dietary treatments according to a double incomplete 4 × 2 Latin square design. The dietary treatments in Exp. 1 were the control diet with water infusion, the control diet with pectin infusion, the MCP diet with water infusion, or the MCP diet with pectin infusion. In Exp. 2, the pigs received the same control treatments as in Exp. 1 and the phytase diet in combination with water or pectin infusion. Gene copy numbers of total bacteria, *Lactobacillus* spp., *Lactobacillus reuteri*, *Lactobacillus amylovorus*/*Lactobacillus sobrius*, *Lactobacillus mucosae*, *Enterococcus* spp., *Enterococcus faecium*, *Enterococcus faecalis*, bifidobacteria, the *Clostridium coccoides* cluster, the *Clostridium leptum* cluster, the *Bacteroides-Prevotella-Porphyrmonas* group, and *Enterobacteriaceae* were determined by quantitative PCR in DNA extracts of ileal digesta. In Exp. 1, addition of MCP reduced ileal gene copy numbers of *Enterococcus* spp. ($P = 0.048$), *E. faecium* ($P = 0.015$), and the *C. leptum* cluster ($P = 0.028$), whereas pectin infusion enhanced ($P = 0.008$) ileal D-lactate concentration. In Exp. 2, supplemental phytase led to greater ileal gene copy numbers of the *C. coccoides* ($P = 0.041$) and *C. leptum* ($P = 0.048$) clusters and the *Bacteroides-Prevotella-Porphyrmonas* group ($P = 0.033$), whereas it reduced ($P = 0.027$) fecal *n*-butyrate concentration. Pectin infusion reduced ($P = 0.005$) ileal gene copy number of the *C. leptum* cluster. In conclusion, ileal bacterial populations and fermentation patterns are susceptible to changes in the intestinal availability of Ca and P as well as to the supply of pectin as a fermentable substrate. Greater intestinal Ca availability decreased the numbers of some gram-positive bacteria, whereas greater P availability in the small intestine caused by phytase activity enhanced the growth of strictly anaerobic bacteria.

Key words: bacteria, calcium phosphate, pectin, phytase, pig, real-time polymerase chain reaction

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INTRODUCTION

The intestinal microbiota and its metabolic activities are considered to be important factors for animal health and performance in growing pigs (Gaskins, 2001). Dietary factors such as type and inclusion level of fermentable carbohydrates (Owusu-Asiedu et al., 2006; Metzler et al., 2009), but also the supply of minerals, including Ca and P, have been shown to be important modulators of microbial fermentation in rats (Ten Bruggencate et al., 2004), ruminants (Komisarczuk et al., 1987), and pigs (Metzler et al., 2008). Because of environmental concerns, there is constant progress to reduce P excretion by reducing the dietary P content in pig diets, supplementing microbial phytase, or introducing reduced-phytate crops into diet formulation (Knowlton et al., 2004; Veum et al., 2007). However, changing the dietary P and Ca supply may affect the bacterial community inhabiting the different segments of the gastrointestinal tract (**GIT**) of the pig, as shown in studies with rats. A greater dietary intake of Ca and P increased the numbers of ileal and fecal lactobacilli and reduced those of *Salmonella enterica* serovar Enteritidis in rats (Ten Bruggencate et al., 2004), but data for pigs are missing. In pigs, bacterial cellulase activity in feces was decreased by dietary phytase addition (Metzler et al., 2008), indicating changes in bacterial composition and activity, particularly with regard to fermentable carbohydrate degradation. Thus, it was hypothesized that a different supply of minerals, such as Ca and P, and fermentable carbohydrates may change the bacterial populations in the small intestine and the fermentation patterns in the ileum and colon of pigs. Therefore, the objective of this study was to determine the effect of different dietary levels of Ca and P and microbial phytase supplementation either without or in combination with an ileal infusion of pectin as a fermentable substrate on the ileal microbiota and on microbial metabolites in ileal digesta and feces.

MATERIALS AND METHODS

The research protocol was approved by the German Ethical Commission of Animal Welfare of the Provincial Government of Baden-Wuerttemberg. Care of the animals used in this experiment was in accordance with the guidelines issued by German Regulation for Care and Treatments of Animals (Lorz and Metzger, 1999).

Animals and Dietary Treatments

The experiment was conducted at the Institute of Animal Nutrition of the University of Hohenheim (Stuttgart, Germany). Pigs in the present study were housed and fed as previously described in detail by Metzler et al. (2008). Briefly, 2 experiments with 8 barrows (German Landrace × Piétrain) each were conducted. At an average BW of 30.7 ± 0.8 kg and 29.5 ± 1.5 kg in Exp. 1 and 2, respectively, the pigs were surgically fitted at the distal ileum with a simple T-cannula made from ultra-high-molecular-weight polyethylene according to the procedures adapted from Li et al. (1993). The pigs were housed individually in stainless steel metabolic crates (0.8 × 1.5 m), and were able to move around freely and have visual contact with each other. Each crate was equipped with a low-pressure drinking nipple, which allowed free access to demineralized water. The room temperature was kept at 22 ± 2°C. During the 10-d recuperation period after surgery, the feed allowance was gradually increased from 100 to 1,000 g/d.

In both experiments, a control diet (Table 1), based on corn and soybean meal, was formulated to meet or exceed nutrient requirements for pigs according to Deutsche Landwirtschaftsgesellschaft (1991) recommendations, but with a daily P supply below the actual requirement of the animal (Metzler et al., 2008). The feedstuffs were ground to pass a 3.0-mm mesh screen before incorporation into the diets. In both experiments, a suspension of highly methylated apple pectin (Apple Pectin Classic AU 202, Herbstreith and Fox KG, Neuenbürg, Germany; P, 0.08%, and Ca, 0.2%) was infused into the ileal cannula by means of a syringe. Before the infusions, pectin was suspended in demineralized water at a ratio of 1:30 (wt/wt) and was kept in a water bath at approximately 40°C before being infused. A total of 60 g of pectin was infused 3 times daily, with equal amounts at 0830, 1330, and 1630 h. The amount of pectin infused was gradually increased from 10 to 60 g/d within the first 8 d of each experimental period. A total of 1.8 L/d of demineralized water was infused as a control treatment into the ileal cannula of pigs that did not receive the pectin infusion.

In Exp. 1, the 4 dietary treatments were the control diet with ileal infusion of water or pectin, and the control diet supplemented with 15 g/kg of monocalcium phosphate (**MCP**) together with ileal infusion of water or pectin. The addition of MCP to the control diet at the expense of cornstarch resulted in dietary P (7.98 g/kg of DM) and Ca (11.49 g/kg of DM) content that exceeded the P and Ca requirements of growing pigs by approximately 60 and 90%, respectively (Deutsche Landwirtschaftsgesellschaft, 1991). In Exp. 2, the 4 dietary treatments included the control treatments of Exp. 1; in addition, the control diet was supplemented with 1,000 phytase units of phytase/kg (where 1 phytase unit is defined as the amount of enzyme that liberates 1 mmol of inorganic P/min from 5.1 m*M* sodium phytate at 37.0°C and pH 5.5; Natuphos, BASF AG, Ludwigshafen, Germany) in combination with ileal infusion of water or pectin. Microbial phytase was added to the control diet at the expense of corn starch. In both experiments, the pigs were fed twice daily, in equal amounts at each meal, at 0700 and 1900 h. The diets were fed at a rate of approximately 2.1 times the maintenance requirement for ME (i.e., 106 kcal/kg of $BW^{0.75}$), which corresponded to 1,000 and 1,200 g/d (as-fed basis) in experimental periods I and II of both experiments, respectively.

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Experimental Design and Collection Procedures

Both experiments were arranged according to a double incomplete (4 treatments × 2 periods) Latin square design. Each dietary treatment was allotted to 2 out of 8 pigs in experimental periods I and II of both experiments, which resulted in 4 observations per treatment. The dietary treatments were randomized among pigs in both experimental periods to balance for simple first-order carryover effects of one treatment into the following treatment period. Each experimental period comprised 22 d, which included an adaptation period of 15 d. During each experimental period, total collection of feces began at 0700 h on d 16 and ended at 0700 h on d 21. Feces were collected using 3-L polyethylene bags and a silicon ring, which was attached at the anal region by means of skin adhesive (Medical Adhesive, Hollister, Libertyville, IL) and sticking plaster. After taking subsamples of fresh feces for the analysis of short-chain fatty acids (**SCFA**), feces were stored at −32°C until analysis. Fecal samples for DNA analysis were collected separately by means of rectal stimulation twice at 1100 h on d 2 and 3 of total feces collection. The samples were stored in sterile 15-mL tubes. Ileal digesta were collected for a total of 24 h during two 12-h intervals: from 0700 to 1900 h on d 21 and from 1900 to 0700 h on d 22. The collection procedure was adapted from the method of Li et al. (1993) by using plastic tubing attached to the barrel of the cannula by elastic bands. Every 2 h, subsamples of ileal digesta of approximately 50 mL were collected for the determination of SCFA and lactate. Subsamples for DNA analysis were taken separately and stored in sterile 15-mL tubes. Samples for DNA analysis of ileal digesta and feces were transferred directly to an ice-water bath for a maximum of 10 min and then immediately stored at −75°C. The subsamples of ileal digesta for SCFA and lactate determination were stored at +4°C until the end of each 12-h digesta collection period. Thereafter, they were pooled within pigs and stored at −32°C until SCFA and lactate analyses.

Table 1. Ingredients and analyzed chemical composition of the control diet in Exp. 1 and 2 (as-fed basis)

Item	Amount
Ingredient, %	
Corn	57.3
Soybean meal	14.0
Cornstarch	10.0
Sugar beet pulp	5.0
Potato protein	5.0
Dried egg white	3.0
Soybean oil	2.0
Limestone	1.2
P-free vitamin-mineral premix[1]	1.0
Dextrose	1.0
DL-Methionine	0.1
L-Tryptophan	0.1
TiO_2	0.3
Analyzed chemical composition, % of DM	
DM	89.3
CP	20.0
Lys	1.1
P	0.3
Ca	0.8
NDF	12.3
ME,[2] Mcal/kg	3.75

[1]Vitamin-mineral premix (BASU-Mineralfutter GmbH, Bad Sulza, Germany) provided per kilogram of diet: vitamin A, 4,000 IU; vitamin D_3, 500 IU; vitamin E, 15 IU; menadione, 150 μg; thiamine, 1.7 mg; riboflavin, 2.5 mg; pyridoxin, 3 mg; cobalamin, 18 μg; pantothenic acid, 10 mg, niacin, 15 mg; folic acid, 0.25 mg; biotin, 20 μg; choline chloride, 500 mg; Ca, 1.5 g; Na, 1 g; Mg, 500 mg; Zn, 100 mg; Fe, 100 mg; Mn, 20 mg; Cu, 6 mg; Co, 75 μg; I, 200 μg; Se, 300 μg.

[2]Calculated according to Deutsche Landwirtschaftsgesellschaft (1991).

Analytical Methods

Real-Time PCR Assays. Total nucleic acids were extracted from ileal and fecal samples according to the method described in detail by Vahjen et al. (2007). In short, 1 g of sample was sheared with glass beads in a 4 *M* guanidinium isothiocyanate solution with a bead beater. After phenol-chloroform extraction, crude nucleic acids were collected by isopropanol precipitation and purified with commercial spin columns (Machery-Nagel, Düren, Germany).

Primer sequences are given in Table 2. All primers were purchased from MWG Biotech (Straubing, Germany). A stratagene MX3000p instrument (Stratagene, Amsterdam, the Netherlands) was used for PCR amplification and fluorescent data collection. The master mix consisted of 12.5 μL of Brilliant SYBR Green QPCR Master Mix (Stratagene) or 12.5 μL of HotStartTaq Master Mix (Qiagen, Hilden, Germany) for Taqman assays, 0.5 μL of each primer (10 μ*M*), 0.75 μL of ROX reference dye (1:500 diluted), and 10.75 μL of water. One microliter of sample was added before PCR amplification. The PCR products with correct melting temperature profiles were randomly (n = 3 per primer pair) checked by agarose gel electrophoresis (2%). Amplification involved 1 cycle at 95°C for 15 min for initial denaturation, followed by 40 cycles of denaturation at 95°C for 15 s, primer annealing at the optimal temperatures (see Table 2) for 30 s, and extension at 72°C for 30 s. Calibration standards were developed using a series of autoclaved sow feces samples as the most complex sample matrix spiked with different bacterial species and known cell numbers (10^9 to 10^3 cells/g of fresh matter), as outlined by Vahjen et al. (2007). The calibration samples consisted of 81 reference and isolate strains, and are given in detail by Vahjen et al. (2007). The strains not mentioned in that study were *Bacteroides fragilis* DSM 2151, *Bacteroides thetaiotaomicron* DSM 2079, *Bacteroides vulgatus* DSM 1447, *Clostridium butyricum* DSM 10702, *Clostridium cellulovorans* DSM 3052, *Clostridium coccoides* DSM 935, and *Clostridium leptum* DSM 752. After extraction and purification, these extracts were used as PCR calibration samples,

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Table 2. The 16S ribosomal DNA real-time PCR primers used to detect bacterial groups and species in intestinal samples

Bacterial group or species	Item	Olionucleotide sequence (5′ to >3′)	A_T[1]	Reference
Total eubacteria	Forward	GGATTAGATACCCTGGTAGTC	50	Lyons et al., 2000
	Reverse	TACCTTGTTACGACTT		
	Probe	FAM-TGACGGGCGGTGTGTACAAGGC-TAM		
Lactobacillus spp.	Forward	AGCAGTAGGGAATCTTCCA	62	Walter et al., 2001
	Reverse	CACCGCTACACATGGAG		Heilig et al., 2002
Enterococcus spp.	Forward	CCCTTATTGTTAGTTGCCATCATT	60	Rinttilä et al., 2004
	Reverse	ACTCGTTGTACTTCCCATTGT		
Bacteroides-Prevotella-Porphyrmonas group	Forward	GGTGTCGGCTTAAGTGCCAT	60	Rinttillä et al., 2004
	Reverse	CGGA(C/T)GTAAGGGCCGTGC		
Enterobacteriaceae	Forward	GTTAATACCTTTGCTCATTGA	60	Malinen et al., 2003
	Reverse	ACCAGGGTATCTAATCCTGTT		
	Probe	(FAM)-CGTGCCAGCAGCCGCGGTA-(DABCYL)		
Bifidobacterium spp.	Forward	TCGCGTC(C/T)GGTGTGAAAG	63	Rinttillä et al., 2004
	Reverse	CCACATCCAGC(A/G)TCCAC		
Clostridium coccoides cluster	Forward	AAATGACGGTACCTGACTAA	58	Matsuki et al., 2002
	Reverse	CTTTGAGTTTCATTCTTGCGAA		
Clostridium leptum cluster	Forward	GCACAAGCAGTGGAGT	60	Matsuki et al., 2004
	Reverse	CTTCCTCCGTTTTGTCAA		
Lactobacillus reuteri[2]	Forward	GCGGTGTGCCTAATACATGC	60	—
	Reverse	TCCATCCCAGAGTGATAGCC		
Lactobacillus amylovorus/Lactobacillus sobrius[2]	Forward	GTATCCCAGACTTAGGGGCAGGT	60	—
	Reverse	GTTTCCAAATGGTATCCCAGACTT		
Lactobacillus mucosae[2]	Forward	GGCTATCACTTTGGGATGGA	60	—
	Reverse	ATGGACCGTGTCTCAGTTCC		
Enterococcus faecalis	Forward	TTCACTGGCTACCTGCTGTG	55	Eaton and Gasson, 2001
	Reverse	AACGCGCCAATTTGTTTTAC		
Enterococcus faecium	Forward	CTTATGATTTGCCAGCAGCA	55	Eaton and Gasson, 2001
	Reverse	TGGATTGTTTCGATGTTCCA		

[1]A_T = annealing temperature (in °C).
[2]Developed at the Institute of Animal Nutrition, Veterinary Faculty of the Free University of Berlin (Berlin, Germany).

and results are expressed as $\log_{10}$ 16S ribosomal DNA gene copies per gram of fresh matter.

Chemical Analyses. For analysis of D- and L- lactic acid, a commercially available photometric test kit (Boehringer, Ingelheim am Rhein, Germany) was used. Short-chain fatty acid concentrations were measured by gas chromatography (HP 6890 Plus GC-System, Hewlett-Packard GmbH, Waldbronn, Germany) using 4-methyl-iso-valerianic acid as the internal standard. The preparation of samples was performed according to the method of Zijlstra et al. (1977). Ileal samples from day and night collection were pooled per animal and homogenized. Samples of diets were finely ground to pass through a 1.0-mm mesh screen (Laboratory Retsch mill, Haan, Germany) before analysis of proximate nutrients, P, and Ca according to the method of the Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (Naumann and Bassler, 1997).

Statistical Analysis

Data were analyzed by ANOVA using the MIXED procedure (SAS Inst. Inc., Cary, NC). Fixed effects included animal and treatment effects. Period and animal within a square were considered random effects, assuming a compound symmetry variance-covariance structure (type = cs). In Exp. 1, orthogonal contrasts were used to test the effects of ileal pectin application (pectin vs. water infusion), MCP addition (MCP vs. control diet), and the interaction of pectin infusion × MCP diet. In Exp. 2, orthogonal contrasts were used to examine the effects of ileal pectin application (pectin vs. water infusion), phytase supplementation (phytase vs. control diet), and the interaction of pectin infusion × phytase diet. Degrees of freedom were approximated using the Kenward-Rogers method (DDFM = kr). A probability level of $P \leq 0.05$ was defined as a significant difference, and trends were discussed up to $P < 0.10$.

RESULTS

Pigs recovered well from surgery and remained healthy throughout the experiments. No feed refusals occurred. The average BW of pigs was 30.7 ± 0.8 kg and 29.5 ± 1.5 kg at surgery and 53.0 ± 2.8 and 52.8 ± 1.9 kg at the conclusion of Exp. 1 and 2, respectively.

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Table 3. Effects of dietary monocalcium phosphate (MCP) supplementation and ileal pectin infusion on total bacteria in ileal digesta and feces and on bacterial populations (log_{10} 16S ribosomal DNA gene copies/g of fresh matter) in the ileum of growing pigs (Exp. 1)

	Treatment[1]					Contrast, *P*-value		
	Control diet		MCP diet					
Item	Water[2] infusion	Pectin[3] infusion	Water infusion	Pectin infusion	SEM	Pectin vs. water infusion	MCP vs. control diet	Pectin infusion × MCP diet
Total bacteria								
Ileum	9.6	10.1	9.2	9.4	0.92	0.778	0.579	0.855
Feces	10.9	11.4	11.8	10.9	0.28	0.549	0.412	0.073
Ileal digesta								
Lactobacillus spp.	10.3	10.0	8.4	9.3	0.77	0.667	0.123	0.767
Lactobacillus reuteri	8.0	7.7	7.4	6.7	0.39	0.222	0.062	0.512
Lactobacillus amylovorus/Lactobacillus sobrius	5.8	5.9	1.9	6.5	1.64	0.164	0.401	0.914
Lactobacillus mucosae	8.3	8.4	7.3	6.6	0.69	0.683	0.069	0.440
Enterococcus spp.	7.3	7.7	7.0	6.7	0.29	0.801	0.048	0.449
Enterococcus faecium	6.4	6.1	5.5	6.2	0.08	0.086	0.015	0.005
Enterococcus faecalis	4.7	7.0	5.7	6.6	0.66	0.084	0.433	0.243
Clostridium coccoides cluster	5.9	5.3	5.3	5.5	5.49	0.823	0.725	0.909
Clostridium leptum cluster	6.2	5.9	4.9	5.0	0.41	0.805	0.028	0.478
Bifidobacteria	5.5	8.6	6.6	6.5	0.79	0.077	0.529	0.077
Bacteroides-Prevotella-Porphyrmonas group	1.7	2.0	2.4	3.2	0.88	0.525	0.289	0.835
Enterobacteriaceae	7.6	7.5	7.7	8.5	0.35	0.291	0.203	0.494

[1]Values are least squares means of 4 observations per treatment.
[2]A total of 1.8 L of demineralized water was infused per day.
[3]Pectin was suspended in 1.8 L of demineralized water at a ratio of 1:30 (wt/wt).

Exp. 1

Bacterial Numbers. Total bacterial populations and *Lactobacillus* spp. in ileal digesta were not affected by pectin infusion ($P = 0.778$) or by addition of MCP ($P = 0.579$) to the diet (Table 3). However, there was a trend ($P = 0.073$) for an interaction of pectin infusion × MCP diet on the gene copy number of total fecal bacteria. Pectin infusion increased the gene copy number of total bacteria in pigs fed the control diet, whereas pectin infusion in combination with supplemental MCP reduced the total bacterial gene copy number. There was a decrease in ileal populations of *Enterococcus* spp. ($P = 0.048$), *Enterococcus faecium* ($P = 0.015$), and the *C. leptum* cluster ($P = 0.028$), and a trend toward reduced gene copy numbers for *Lactobacillus reuteri* ($P = 0.062$) and *Lactobacillus mucosae* ($P = 0.069$) with MCP in the diet. Moreover, pectin infusion tended to increase the ileal gene copy numbers of *E. faecium* ($P = 0.086$), *Enterococcus faecalis* ($P = 0.084$), and bifidobacteria ($P = 0.077$). There was an interaction of pectin infusion and MCP diet for the gene copy number of *E. faecium* ($P = 0.005$). For pigs fed the control diet, pectin infusion reduced the gene copy number of *E. faecium*, but *E. faecium* was increased when the pigs were fed the MCP diet. There was also a trend ($P = 0.077$) toward an interaction of pectin infusion and MCP addition on the gene copy number of bifidobacteria. Pectin infusion increased the number of bifidobacteria in pigs fed the control diet, but decreased bifidobacteria in pigs fed the MCP diet. The gene copy number of *Enterobacteriaceae* was not affected by either MCP addition ($P > 0.203$) or pectin infusion ($P > 0.291$).

Microbial Metabolites. Dry matter content of ileal digesta was not affected by dietary MCP supplementation ($P > 0.383$) or pectin infusion ($P > 0.806$; Table 4). Similarly, addition of MCP ($P > 0.285$) and pectin infusion ($P > 0.667$) did not affect ileal SCFA concentrations. A greater molar proportion ($P = 0.031$) of acetate was measured in ileal digesta samples of pigs fed the MCP diet. Infusion of pectin increased ($P = 0.008$) ileal D-lactate levels and tended ($P = 0.082$) to increase the molar proportion of isobutyrate. In feces, pectin infusion increased ($P = 0.045$) *n*-valerate concentration and tended ($P = 0.096$) to increase its molar proportion (Table 5). Similarly, pectin tended to increase concentrations of total SCFA ($P = 0.072$) and acetate ($P = 0.064$) in feces.

Exp. 2

Bacterial Numbers. There was a trend ($P = 0.071$) for an interaction of pectin infusion and phytase addition to the diet on total bacteria in ileal digesta (Table 6). Pectin infusion decreased the gene copy number of total bacteria in pigs fed the control diet but enhanced the gene copy number of total bacteria when phytase was added to the diet. In contrast, total bacterial populations in feces tended ($P = 0.076$) to be reduced by phytase supplementation. There were no effects of phytase diet ($P = 0.549$ to $P = 0.961$) or pectin infusion ($P = 0.683$ to $P = 0.754$) on gene

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Table 4. Effects of dietary monocalcium phosphate (MCP) supplementation and ileal pectin infusion on DM content (g/kg), concentrations of lactate (µmol/g of fresh matter), and concentrations and molar proportions of short-chain fatty acids (SCFA; µmol/g of fresh matter) in ileal digesta of growing pigs (Exp. 1)

	Treatment[1]					Contrast, *P*-value		
	Control diet		MCP diet					
Item	Water[2] infusion	Pectin[3] infusion	Water infusion	Pectin infusion	SEM	Pectin vs. water infusion	MCP vs. control diet	Pectin infusion × MCP diet
DM, g/kg	10.7	10.7	10.2	10.5	0.43	0.806	0.383	0.768
Concentration, µmol/g of fresh matter								
D- + L-Lactate	42.5	42.5	22.0	34.7	11.04	0.667	0.285	0.616
D-Lactate	4.5	13.7	0.7	13.0	1.95	0.008	0.325	0.487
L-Lactate	37.9	28.9	21.3	21.8	11.96	0.774	0.359	0.699
Total SCFA	33.7	35.7	47.6	38.4	9.96	0.734	0.441	0.431
Acetate	31.1	29.7	44.1	27.1	8.98	0.468	0.612	0.455
Propionate	4.1	3.5	4.3	2.3	0.80	0.236	0.556	0.416
n-Butyrate	1.9	1.6	2.7	0.8	0.77	0.325	0.979	0.362
Isobutyrate	0.2	0.2	0.3	0.2	0.08	0.425	0.581	0.430
n-Valerate	ND[4]	ND	ND	ND	—	—	—	—
Isovalerate	0.3	0.4	0.6	0.3	0.15	0.739	0.324	0.216
SCFA molar proportion, %								
Acetate	82.7	83.8	85.2	87.9	11.53	0.224	0.031	0.489
Propionate	12.3	10.5	8.9	7.4	1.71	0.449	0.106	0.954
n-Butyrate	4.2	4.3	4.3	3.4	0.74	0.673	0.647	0.487
Isobutyrate	0.4	0.5	0.4	0.5	0.03	0.082	0.369	0.126
n-Valerate	ND	ND	ND	ND	—	—	—	—
Isovalerate	0.6	0.9	1.0	1.0	0.13	0.487	0.166	0.254

[1]Values are least squares means of 4 observations per treatment.
[2]A total of 1.8 L of demineralized water was infused per day.
[3]Pectin was suspended in 1.8 L of demineralized water at a ratio of 1:30 (wt/wt).
[4]ND = below detection level.

Table 5. Effects of dietary monocalcium phosphate (MCP) supplementation and ileal pectin infusion on DM content (g/kg) and on concentrations and molar proportions of short-chain fatty acids (SCFA; µmol/g of fresh matter) in feces of growing pigs (Exp. 1)

	Treatment[1]					Contrast, *P*-value		
	Control diet		MCP diet					
Item	Water[2] infusion	Pectin[3] infusion	Water infusion	Pectin infusion	SEM	Pectin vs. water infusion	MCP vs. control diet	Pectin infusion × MCP diet
DM, g/kg	36.7	35.9	38.8	34.6	1.59	0.247	0.816	0.412
Concentration, µmol/g of fresh matter								
Total SCFA	103.1	138.5	108.1	130.7	10.82	0.072	0.887	0.983
Acetate	57.5	79.7	64.1	75.1	5.88	0.064	0.867	0.374
Propionate	22.5	27.8	22.3	27.0	2.14	0.131	0.850	0.885
n-Butyrate	11.9	15.6	10.3	14.2	1.73	0.151	0.462	0.958
Isobutyrate	2.9	4.0	3.1	3.8	0.47	0.176	0.947	0.724
n-Valerate	2.9	4.8	3.0	4.3	0.49	0.045	0.683	0.602
Isovalerate	5.4	7.1	5.4	6.3	1.07	0.340	0.712	0.738
SCFA molar proportion, %								
Acetate	56.3	57.2	59.8	57.5	1.53	0.712	0.260	0.331
Propionate	21.8	20.1	20.4	20.9	0.75	0.542	0.731	0.205
n-Butyrate	11.2	11.2	9.2	10.6	1.02	0.606	0.261	0.534
Isobutyrate	2.8	2.9	2.8	2.9	0.26	0.824	0.915	0.970
n-Valerate	2.9	3.4	2.7	3.2	0.22	0.096	0.540	0.982
Isovalerate	5.1	5.2	4.9	4.8	0.63	0.985	0.685	0.885

[1]Values are least squares means of 4 observations per treatment.
[2]A total of 1.8 L of demineralized water was infused per day.
[3]Pectin was suspended in 1.8 L of demineralized water at a ratio of 1:30 (wt/wt).

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Table 6. Effects of dietary phytase supplementation and ileal pectin infusion on total bacteria in ileal digesta and feces and bacterial populations ($\log_{10}$ 16S ribosomal DNA gene copies/g of fresh matter) in the ileum of growing pigs (Exp. 2)

	Treatment[1]					Contrast, P-value		
	Control diet		Phytase diet					
Item	Water[2] infusion	Pectin[3] infusion	Water infusion	Pectin infusion	SEM	Pectin vs. water infusion	Phytase vs. control diet	Pectin infusion × phytase diet
Total bacteria								
Ileum	9.9	8.3	9.3	10.9	0.71	0.978	0.220	0.071
Feces	11.9	11.7	10.7	11.3	0.55	0.768	0.076	0.313
Ileal digesta								
Lactobacillus spp.	10.4	10.2	9.9	10.6	0.54	0.698	0.892	0.366
Lactobacillus reuteri	7.2	7.3	7.2	7.4	0.47	0.754	0.961	0.873
Lactobacillus amylovorus/Lactobacillus sobrius	5.6	5.6	3.9	5.4	1.47	0.683	0.549	0.625
Lactobacillus mucosae	8.8	8.3	8.4	8.3	0.73	0.736	0.822	0.774
Enterococcus spp.	7.3	6.2	6.4	7.1	0.38	0.654	0.924	0.061
Enterococcus faecium	6.3	6.2	5.1	5.8	0.12	0.045	0.002	0.146
Enterococcus faecalis	4.9	5.7	7.2	5.4	0.39	0.217	0.047	0.159
Clostridium coccoides cluster	5.0	3.9	5.9	5.3	0.49	0.117	0.041	0.925
Clostridium leptum cluster	5.6	3.7	6.3	5.5	0.33	0.005	0.048	0.126
Bifidobacteria	8.4	7.8	7.5	7.9	0.24	0.726	0.135	0.126
Bacteroides-Prevotella-Porphyrmonas	2.9	1.0	3.7	3.4	0.54	0.061	0.033	0.232
Enterobacteriaceae	6.9	5.7	6.6	8.1	0.43	0.718	0.071	0.062

[1]Values are least squares means of 4 observations per treatment.
[2]A total of 1.8 L of demineralized water was infused per day.
[3]Pectin was suspended in 1.8 L of demineralized water at a ratio of 1:30 (wt/wt).

copy numbers of the lactobacillus group and species as well as on *Enterococcus* spp. in ileal digesta. However, supplemental phytase decreased ($P = 0.002$) the gene copy number of *E. faecium*, whereas pectin increased ($P = 0.045$) the number of *E. faecium.* Supplementation of phytase increased the gene copy number of *E. faecalis* ($P = 0.047$) and those of the *C. coccoides* ($P = 0.041$) and *C. leptum* ($P = 0.048$) clusters and the *Bacteroides-Prevotella-Porphyrmonas* group ($P = 0.033$). Moreover, phytase tended ($P = 0.071$) to increase the gene copy number of *Enterobacteriaceae*. Pectin infusion, in turn, decreased ($P = 0.005$) the gene copy number of the *C. leptum* cluster and tended to decrease the gene copy number of the *Bacteroides-Prevotella-Porphyrmonas* group ($P = 0.061$). Furthermore, there was a trend ($P = 0.062$) for an interaction of pectin infusion × phytase on the gene copy number of *Enterobacteriaceae.* Pectin infusion in pigs fed the control diet tended to cause a smaller gene copy number of *Enterobacteriaceae*, but with a trend toward greater numbers after phytase supplementation.

Microbial Metabolites. Dry matter content of ileal digesta and feces and of ileal concentrations of SCFA and lactate were not affected by dietary phytase supplementation ($P = 0.111$ to $P = 0.972$) and ileal pectin infusion ($P = 0.150$ to $P = 0.989$; Table 7). In contrast, supplemental phytase reduced fecal concentration of n-butyrate ($P = 0.027$) and its molar proportion ($P = 0.039$; Table 8). Similarly, concentrations of total SCFA ($P = 0.080$), isobutyrate ($P = 0.080$), and isovalerate ($P = 0.054$) tended to be less in feces of pigs fed the phytase diet.

DISCUSSION

Bacterial Numbers

Feeding of low-P diets and dietary supplementation of exogenous microbial phytase to further improve P utilization are frequently practiced in pig production. However, little information is available on the response of the bacterial community inhabiting the GIT of pigs to differences in intestinal Ca and P availability caused by variations in dietary Ca and P supplies. In the present study, the ileum was targeted to study the effects of different dietary Ca and P supplies and of phytase supplementation as the terminus of the major area of nutrient absorption in the pig. There, microbial communities are less complex than in more distal regions, and biologically relevant effects of diet might be more easily identified (Hill et al., 2005). Overall, estimated values for total bacteria, *Lactobacillus* spp., *Enterococcus* spp., bifidobacteria, the *C. coccoides* and *C. leptum* clusters, the *Bacteroides-Prevotella-Porphyrmonas* group, and *Enterobacteriaceae* in ileal digesta samples were in the range of those described previously (e.g., Branner et al., 2004; Castillo et al., 2008; Metzler et al., 2009). However, a 2.7- and 1.4-fold increase in total dietary P and Ca content, respectively, after MCP supplementation of the control diet reduced the pro-

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Table 7. Effects of dietary phytase supplementation and ileal pectin infusion on DM content (g/kg), concentrations of lactate (µmol/g of fresh matter), and concentrations and molar proportions of short-chain fatty acids (SCFA; µmol/g of fresh matter) in ileal digesta of growing pigs (Exp. 2)

	Treatment[1]					Contrast, *P*-value		
	Control diet		Phytase diet					
Item	Water[2] infusion	Pectin[3] infusion	Water infusion	Pectin infusion	SEM	Pectin vs. water infusion	Phytase vs. control diet	Pectin infusion × phytase diet
DM, g/kg	9.7	11.0	8.7	9.8	0.73	0.150	0.111	0.911
Concentration, µmol/g of fresh matter								
D- + L-Lactate	43.8	35.7	35.3	42.7	3.85	0.954	0.851	0.092
D-Lactate	8.5	8.4	4.5	9.2	1.89	0.357	0.415	0.253
L-Lactate	35.2	27.3	30.7	33.6	3.16	0.527	0.784	0.138
Total SCFA	28.1	30.8	31.1	26.9	3.03	0.806	0.884	0.288
Acetate	22.8	27.5	26	21.4	3.63	0.989	0.696	0.241
Propionate	2.8	2.6	2.9	2.1	0.47	0.423	0.677	0.557
n-Butyrate	1.6	1.8	1.7	1.7	0.41	0.790	0.972	0.862
Isobutyrate	0.1	0.1	0.1	0.1	0.02	0.767	0.871	0.392
n-Valerate	0.2	0.2	0.2	0.2	0.06	0.885	0.977	0.474
Isovalerate	0.2	0.2	0.2	0.2	0.02	0.437	0.605	0.270
SCFA molar proportion, %								
Acetate	82.1	85.3	83.6	83.3	1.31	0.392	0.838	0.229
Propionate	10.4	7.9	9.5	8.3	1.10	0.218	0.811	0.559
n-Butyrate	6.0	5.4	5.4	6.6	0.70	0.741	0.626	0.245
Isobutyrate	0.3	0.3	0.3	0.3	0.06	0.891	0.619	0.910
n-Valerate	0.7	0.7	0.8	0.8	0.16	0.960	0.631	0.919
Isovalerate	0.6	0.5	0.5	0.7	0.11	0.525	0.479	0.107

[1]Values are least squares means of 4 observations per treatment.
[2]A total of 1.8 L of demineralized water was infused per day.
[3]Pectin was suspended in 1.8 L of demineralized water at a ratio of 1:30 (wt/wt).

Table 8. Effects of dietary phytase supplementation and ileal pectin infusion on DM content (g/kg) and on concentrations and molar proportions of short-chain fatty acids (SCFA; µmol/g of fresh matter) in feces of growing pigs (Exp. 2)

	Treatment[1]					Contrast, *P*-value		
	Control diet		Phytase diet					
Item	Water[2] infusion	Pectin[3] infusion	Water infusion	Pectin infusion	SEM	Pectin vs. water infusion	Phytase vs. control diet	Pectin infusion × phytase diet
DM, g/kg	38.6	35.6	35.8	34.9	1.09	0.143	0.112	0.244
Concentration, µmol/g of fresh matter								
Total SCFA	126.9	120.1	104.9	95.3	11.03	0.561	0.080	0.901
Acetate	68.3	65.2	62.3	56.3	5.71	0.531	0.236	0.800
Propionate	23.6	25.9	19.2	16.5	3.57	0.889	0.118	0.448
n-Butyrate	17.4	12.7	9.8	8.4	1.98	0.255	0.027	0.423
Isobutyrate	4.2	3.8	3.2	3.3	0.34	0.728	0.080	0.556
n-Valerate	5.8	4.6	3.7	4.7	1.06	0.923	0.350	0.345
Isovalerate	7.6	8.0	5.9	6.2	0.70	0.700	0.054	0.955
SCFA molar proportion, %								
Acetate	53.8	54.5	59.7	59.6	2.70	0.930	0.092	0.885
Propionate	18.2	21.3	18.8	17.3	1.46	0.183	0.329	0.158
n-Butyrate	13.9	10.6	8.9	8.7	1.28	0.312	0.039	0.264
Isobutyrate	3.3	3.2	3.1	3.4	0.20	0.835	0.916	0.317
n-Valerate	4.8	3.7	3.6	4.7	0.71	0.960	0.922	0.169
Isovalerate	6.0	6.7	5.9	6.3	0.79	0.590	0.776	0.856

[1]Values are least squares means of 4 observations per treatment.
[2]A total of 1.8 L of demineralized water was infused per day.
[3]Pectin was suspended in 1.8 L of demineralized water at a ratio of 1:30 (wt/wt).

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liferation of *Enterococcus* spp., *E. faecium*, and the *C. leptum* cluster and, as a tendency, decreased the numbers of *L. reuteri* and *L. mucosae* determined at the distal ileum, but total bacterial numbers were not affected. Thus, it can be concluded from the present results that the increase in dietary calcium phosphate content up to 150% of the requirement of growing pigs (Deutsche Landwirtschaftsgesellschaft, 1991) may inhibit the proliferation of specific bacterial species in the upper GIT of pigs when compared with pigs fed the control diet marginal in P. Previously, it has been documented that calcium phosphate in mammalian saliva protects against bacterial overgrowth in dental mucus (Hicks et al., 2003; Becker, 2005). However, in rats an increase in dietary calcium phosphate content tended to promote the growth of ileal lactobacilli by 0.4 to 0.6 log units (Bovee-Oudenhoven et al., 1999) and significantly increased cell counts of fecal lactobacilli by 0.9 to 1.6 log units (Ten Bruggencate et al., 2004). The authors attributed this effect to the ability of the calcium phosphate complex to precipitate cytotoxic fatty and secondary bile acids because these are known to inhibit the growth of various intestinal bacteria, including lactobacilli (Kurdi et al., 2006). However, it has to be emphasized that bile acids originating from rats (tauro-conjugated bile) and pigs (glyco-conjugated bile) differ in the way they bind to calcium phosphate (Van der Meer and De Vries, 1985); this may explain, in part, the different results obtained in rats (Bovee-Oudenhoven et al., 1999; Ten Bruggencate et al., 2004) and in the present study with pigs.

The addition of phytase to the diet did not affect the gene copy numbers of enterococci and lactobacilli in ileal digesta, although the intestinal availability of phytate-bound P, but not that of Ca, was enhanced (Metzler et al., 2008). These findings suggest that dietary Ca, rather than P, may act as a growth-inhibiting factor for specific intestinal bacteria. Increasing the concentration of free Ca ions may reduce the adhesion potential of specific bacterial species, resulting in decreased colonization of mucosal areas because of competition for the same binding sides with other bacteria species, as recently demonstrated in vitro for *Lactobacillus* spp. (Larsen et al., 2007). Bacterial adhesion to the intestinal mucosa is considered an important factor for bacterial colonization because it prevents washout of bacteria, especially in the small intestine, where digesta flow rates are relatively fast (Erickson et al., 1992; Rojas and Conway, 1996). Moreover, addition of Ca inhibited intercellular adhesion of and biofilm formation by cocci such as *Staphylococcus aureus* under in vitro conditions (Arrizubieta et al., 2004). In fact, several cell wall components of gram-positive bacteria, including proteins, exopolysaccharides, and lipoteichoic acid, bind to Ca (Rose, 2000; Ridgen et al., 2003). Accordingly, in the present study, MCP supplementation of the pig diet reduced mainly gram-positive bacteria, such as lactobacilli, enterococci, and members of the *C. leptum* cluster. Calcium is known to promote nonspecific interactions such as neutralization of the electrical double layer between bacterial cells as well as specific adhesive interactions with protein and polysaccharide adhesion molecules at the cell surface (Geesey et al., 2000). Because dietary Ca induces gastric acid secretion by stimulating gastrin release (Floor et al., 1991), it cannot be ruled out that the resulting decrease in gastric pH may have inhibited the adhesion of lactobacilli to the stomach mucosa as well. In fact, Ouwehand et al. (2001) reported that adhesion of the probiotic strains *Lactobacillus brevis* PEL 1 and *L. reuteri* ING1 to intestinal mucus was reduced by exposure to low pH in vitro.

Addition of phytase increased the ileal numbers of strictly anaerobic bacteria, such as the *C. coccoides* cluster, the *C. leptum* cluster, and the *Bacteroides-Prevotella-Porphyrmonas* group, and tended to enhance those of *Enterobacteriaceae.* These findings might be associated with the greater availability of phytate-bound P in the small intestine (Metzler et al., 2008). Moreover, the gene copy number of *Enterococcus* spp. was not affected by addition of phytase; however, the number of *E. faecium* was less, whereas those of *E. faecalis* populations were greater compared with the control treatment.

Interestingly, ileal infusion of pectin altered the growth of some of the bacterial groups and species investigated in ileal digesta. In fact, there was a trend toward greater gene copy numbers, amounting to 0.3 to 1.6 log units for *E. faecium*, *E. faecalis*, and bifidobacteria in the MCP experiment (Exp. 1) and for *E. faecium* in the phytase experiment (Exp. 2), whereas the gene copy number of the *C. leptum* cluster was reduced and those of the *Bacteroides-Prevotella-Porphyrmonas* group tended to be reduced by 1.1 to1.4 log units in the phytase experiment (Exp. 2). These changes in the composition of the bacterial community may be because the passage of digesta from the ileum to the cecum is regulated through the ileocecal sphincter. In fact, emptying of the distal ileum into the cecum is associated with propulsive phasic contractions that are regulated by stimulants in digesta (e.g., SCFA; Fich et al., 1989). Thus, there may have been sufficient time for the bacteria to hydrolyze at least part of the infused pectin, thereby altering the composition of bacterial groups at the distal ileum. However, studies with intact pigs have shown a physiological ceco-ileal reflux occurring approximately 8 times per hour, which may induce a natural contamination of ileal contents by the cecal microbiota (Cuche and Malbert, 1998). This may also explain the observed changes in composition of the bacterial community at the distal ileum of pigs. Generally, pectin is hydrolyzed by different intestinal bacterial groups, including *Bacteroides* spp., clostridia, and bifidobacteria (Dongowski et al., 2002; Olano-Martin et al., 2002). This is in agreement with the trend of increased ileal numbers of bifidobacteria in the MCP experiment (Exp. 1), but not in the phytase experiment (Exp. 2). In contrast, pectin infusion tended to reduce

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the *Bacteroides*-*Prevotella*-*Porphyrmonas* group and decreased the *C. leptum* cluster in the phytase experiment (Exp. 2), but not in the MCP experiment (Exp. 1). The discrepancy among the results of this study and in the literature (Dongowski et al., 2002; Olano-Martin et al., 2002) may be due to differences in the degree of methylation of the pectin sources used in these experiments. Accordingly, Dongowski et al. (2002) used citrus pectin with a degree of methylation in the range of 34 to 93% in studies with rats and in vitro, whereas Olano-Martin et al. (2002) examined low- and highly methylated apple pectin with a degree of methylation of 8 and 66% under in vitro conditions, respectively. Comparison of the results of both studies revealed that pectin-degrading bacteria generally grow better on low-methylated pectin. In the present study, according to the specification of the manufacturer, highly methylated apple pectin with a degree of methylation of 68 to 72% was used, likely slowing its bacterial breakdown (Olano-Martin et al., 2002). The gene copy number of *E. faecium* tended to rise when pectin was ileally infused. This is in contrast to recent findings (Metzler et al., 2009) in which the numbers of *E. faecium* decreased when 25% pectin was included in the diet. To our knowledge, no galacturonase activity has been reported for *E. faecium* to date; therefore, other pectin-degrading bacteria, such as clostridia, *Bacteroides*-like bacteria, and bifidobacteria, and lactobacilli, such as *Lactobacillus sobrius* (Olano-Martin et al., 2002; Konstantinov et al., 2005), may have hydrolyzed pectin, with *E. faecium* strains being able to utilize these hydrolysis products.

Dietary MCP addition, phytase supplementation, or infusion of pectin into the distal ileum did not affect the gene copy number of *Enterobacteriaceae.* This is in agreement with the results of a recent study by Metzler et al. (2009), which demonstrated that inclusion of 25% highly methylated apple pectin to a low-P corn- and soybean meal-based diet did not affect the number of *Enterobacteriaceae.* Similarly, Högberg et al. (2004) failed to detect changes in the diversity of coliform populations in the distal ileum after including soluble and insoluble nonstarch polysaccharides in diets for pigs. However, when culturing techniques were used, soluble fermentable carbohydrates such as guar gum have previously been reported to stimulate the growth of *Enterobacteriaceae* in pigs (Durmic et al., 1998; Owusu-Asiedu et al., 2006).

Although molecular techniques have been widely accepted for the analysis of intestinal bacterial populations, results need to be interpreted with caution. For instance, the mean values for the gene copy number of total eubacteria in the present study were exceeded by the mean values for *Lactobacillus* spp. gene copy number in pigs receiving the control treatments in the MCP experiment (Exp. 1) and for all treatments in the phytase experiment (Exp. 2). This is likely related to the type of assay used because the amplification of gene copies for eubacteria was based on probe hybridization, whereas the *Lactobacillus* spp. group was measured by primer annealing. Some *Lactobacillus* spp. species may have steric hindrance at the point of probe hybridization, which may lead to weaker fluorescence signals than by primer annealing. Moreover, real-time PCR is biased by DNA from dead bacteria that are amplified and hence quantified, and by the multiplicity of 16S ribosomal RNA genes per genome in bacteria (Fogel et al., 1999). Overall, differences between the 2 experiments may be attributed to variations in the bacterial community among individual pigs, which are strongly homeostatic and characteristic for the host from which the digesta sample was obtained (Zoetendal et al., 1998; Hill et al., 2005).

Microbial Metabolites

Dietary supplementation of MCP and phytase as well as ileal infusion of pectin had no effect on ileal SCFA concentrations. However, the increased molar proportion of acetate to total SCFA in the distal ileum of pigs fed the MCP diet may indicate changes in microbial fermentative activity because of the greater Ca and P supplies. Pectin infusion enhanced ileal D-lactate recovery in the MCP experiment (Exp. 1), but not in the phytase experiment (Exp. 2), suggesting changes in absorption of lactate because of altered digesta viscosity, the metabolic activity of lactic acid bacteria, or the metabolic activity of bacteria using lactic acid. At the fecal level, *n*-valerate concentrations were enhanced, and there was a trend toward greater concentrations of acetate in the MCP experiment (Exp. 1), resulting in a trend toward greater total SCFA when pectin was infused as a fermentable substrate. The molar SCFA proportions, in turn, were not different between the diets, although pectin is known as an important precursor of acetate (Macfarlane and Macfarlane, 2003).

Dietary phytase addition reduced microbial butyrate concentrations in the large intestine because both fecal *n*-butyrate concentration and its molar proportion to total SCFA in feces were significantly decreased in pigs fed the phytase diet compared with control animals. This is in agreement with the trend toward reduced concentrations of total SCFA, branched-chain fatty acids, and gene copy number of total bacteria in the feces of pigs fed the phytase diet. There is evidence that because of the release of phytate-bound P, the absorption of P up to the distal ileum is improved, thereby reducing the amount of P entering the large intestine (Metzler et al., 2008). Consequently, the P availability for bacterial assimilation in the large intestine is decreased. In this context, the present findings may be in accordance with observations made in ruminants where P depletion reduces the fermentative activity and growth of rumen bacteria, resulting in decreased production of SCFA and bacterial protein synthesis; the cellulolytic and hemicellulolytic flora are particularly affected (Durand and Komisarczuk, 1988; Wider, 2005). Finally, it should be mentioned that the assessment of true metabolic activity (i.e., determination of SCFA and lactate)

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is biased because fermentation products such as lactate or acetate may act as substrates, thereby modifying the concentrations of these fermentation products. Moreover, 95% of the SCFA produced are absorbed from the large intestine during its transit through the gut (Cummings, 1981).

In conclusion, results of the present study indicate that the composition and metabolic activity of the intestinal microbiota are susceptible not only to fermentable carbohydrates, but also to changes in the intestinal availability of Ca and P because of differences in dietary Ca and P supplies. It appears that an increase in the amount of intestinal Ca may reduce the population densities of some specific bacterial species belonging to lactobacilli, enterococci, and the *C. leptum* cluster, whereas greater intestinal phytate-P availability in the small intestine after phytase supplementation appears to stimulate the growth of strictly anaerobic bacteria. Butyrate fermentation in the large intestine appeared to be less in pigs fed the phytase-supplemented low-P diet. Because of the important role of butyrate in stimulating the proliferation of epithelial cells, mucus release, and water and mineral absorption in the large intestine (Tsukahara et al., 2006), further research is warranted to elucidate whether butyrate-producing bacteria are affected by reduced P content in the large intestine.

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