Published December 5, 2014

The effect of dietary phosphorus and calcium level, phytase supplementation, and ileal infusion of pectin on the chemical composition and carbohydrase activity of fecal bacteria and the level of microbial metabolites in the gastrointestinal tract of pigs[1,2]

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ABSTRACT: Two experiments with growing pigs were conducted to determine the effects of dietary P and Ca level, phytase supplementation, and ileal pectin infusion on ileal and fecal P and Ca balance, chemical composition of fecal mixed bacterial mass (MBM), and bacterial metabolic activity. Pigs (initial BW = 30 kg) were fitted with simple T-cannulas at the distal ileum. They were fed a low-P corn-soybean meal control diet (3 g of P/kg) or the control diet supplemented with monocalcium phosphate (MCP; 7 g of P/kg; Exp. 1) or 1,000 FTU phytase/kg (Exp. 2). The daily infusion treatments consisted of 60 g of pectin dissolved in 1.8 L of demineralized water or 1.8 L of demineralized water as the 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. The dietary treatments in Exp. 1 were the control (Con–) diet with water infusion; the control (Con+) diet with pectin infusion; the MCP diet with water infusion; and the MCP diet with pectin infusion. In Exp. 2, the pigs received the same Con– and Con+ treatments as in Exp. 1 and, in addition, the phytase-supplemented diet in combination with water or pectin infusion. After a 15-d adaptation period, feces were collected for 5 d followed by ileal digesta collection for 24 h. In Exp. 1, supplemental MCP increased ($P \leq 0.003$) ileal and fecal P and Ca recovery as well as P and Ca content of the MBM. Pectin infusion increased the N content of the MBM ($P = 0.054$) and polygalacturonase activity ($P = 0.032$) in feces. In addition, pectin decreased ($P = 0.049$) ileal and tended ($P < 0.079$) to increase fecal VFA concentrations. In Exp. 2, phytase decreased ileal and fecal P recovery ($P < 0.001$) and the P content of the MBM ($P = 0.045$), whereas the N content of the MBM ($P = 0.094$) and fecal cellulase activity ($P = 0.089$) tended to decrease. Similarly, pectin infusion decreased ($P = 0.036$) fecal cellulase activity but increased ($P < 0.001$) polygalacturonase activity. In conclusion, these data indicate that bacterial P and Ca assimilation and metabolic activity depend on P and Ca availability in the large intestine and on the availability of fermentable substrate, such as pectin. Thus, increasing dietary P and Ca levels increases bacterial P and Ca assimilation due to greater intestinal P and Ca availability, whereas decreasing intestinal P availability for bacteria through phytase addition to low-P diets reduces bacterial P incorporation and seems to decrease bacterial activity.

Key words: bacteria, large intestine, pectin, phosphorus, phytase, pig

J. Anim. Sci. 2008. 86:1544–1555
doi:10.2527/jas.2007-0267

INTRODUCTION

In recent years, the microflora present in the gastrointestinal tract (**GIT**) of pigs has been identified as one of the main factors affecting health status and growth performance of growing pigs (Gaskins, 2001). In rats and ruminants, some essential minerals, such as Ca and P, have been proven to be important modula-

[1]This work was supported in part by grants of the German Research Foundation (MO 1406/16-1 and RO 1217/5-1) and BASF (Ludwigshafen, Germany). The German National Academic Foundation is gratefully acknowledged for providing a scholarship to B. U. Metzler. Appreciation is extended to H. Brehm and M. Stefflof the Animal Clinic for performing intestinal surgery, to H. P. Piepho of the section of bioinformatics, and to Q. Zebeli of the Institute of Animal Nutrition for support with statistics and Q. G. Ma of the China Agriculture University (Beijing, China) for assistance in the analysis of amino acids.

[2]Presented in part at the 61st Conference of the Society of Nutrition Physiology in Göttingen, Germany, March 6 to 8, 2007; and 2007 Joint Annual Meeting ADSA-PSA-AMPA-ASAS, July 8 to 12, 2007, San Antonio, TX.

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Received May 15, 2007.
Accepted March 5, 2008.

tors of microbial fermentation (Durand and Komisarczuk, 1988; Bovee-Oudenhoven et al., 1997). According to in vitro studies with rumen bacteria, there exists a minimal P requirement for the fermentation of carbohydrates (Komisarczuk et al., 1987b). However, little is known about the bacterial P requirement in support of fermentation in the large intestine (**LI**) of pigs. Because diets for pigs contain variable amounts of carbohydrates, fermentable substrate differing in type, level, and origin is available for microbial fermentation in the LI. Due to environmental concerns, there is constant progress to decrease P excretion by reducing the dietary P level in pig diets, supplementing microbial phytase or introducing low-phytate crops into diet formulation (Knowlton et al., 2004; Veum et al., 2007). However, reducing the dietary P level may influence the fermentation of carbohydrates. Thus, it was hypothesized that both the dietary P supply and the provision of fermentable substrate in the GIT may affect microbial activity, thereby influencing the utilization of P by intestinal bacteria.

Because monocalcium phosphate (**MCP**) was chosen as highly available source of P, the effects of P and Ca could not be seen separately in the present study. Therefore, the objective of this study was to determine the effect of differences in dietary P and Ca supply and microbial phytase supplementation in combination with or without an ileal infusion of pectin as fermentable substrate on the chemical composition and carbohydrase activity of fecal bacteria, on ileal and fecal P and Ca recoveries as well as on the concentration of 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).The experiment was conducted at the Institute of Animal Nutrition of the University of Hohenheim (Stuttgart, Germany).

Animals and Housing

Two experiments with 8 barrows (German Landrace × Piétrain) each were conducted. The animals, with an average initial BW of 22.4 ± 1.4 and 25.4 ± 1.8 kg in Exp. 1 and 2, respectively, were obtained from the production herd of the Research Station Unterer Lindenhof of the University of Hohenheim (Stuttgart, Germany). The barrows were obtained 3 and 2 wk before the beginning of Exp. 1 and 2, respectively, to allow for adaptation to the housing conditions. They were housed individually in stainless steel metabolic crates (0.8 × 1.5 m). The barrows were able to move freely and had 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 adaptation period to the crates, the barrows were fed ad libitum a 20% CP (as-fed basis) grower diet. At an average BW of 30 kg (Exp. 1, 30.7 ± 0.8 kg; Exp. 2, 29.5 ± 1.5 kg), the barrows were surgically fitted with a simple T-cannula at the distal ileum, according to the procedures adapted from Li (1992), with the following modifications: the cannulas were made from ultra-high-molecular-weight polyethylene with an i.d. of 2.0 cm and wings 11.0 cm long and 2.6 cm wide. The retaining ring had a diameter of 7 cm. During the experiments, the skin around the cannula was cleaned with lukewarm water several times daily, dried, and treated with a skin-protecting paste (Stomahesive Paste, Convatec, Princeton, UK). Additionally, foamed material was put between the retaining ring and the skin to absorb leaking digesta to prevent erythema. The barrows were allowed a recuperation period of 10 d after surgery. During this period, the feed allowance was gradually increased from 100 to 1,000 g/d.

Dietary Treatments

In both experiments, a control diet (Table 1), based on corn and soybean meal, was formulated to meet or to exceed the nutrient requirements for barrows, according to DLG (1991) recommendations, but with a daily P supply below the actual requirement. Therefore, the apparent P absorption was not affected by intestinal homeostasis; otherwise, with increasing levels of dietary P, increasing amounts of P will be excluded from absorption by homeostatic regulatory mechanisms (Zimmermann et al., 2002). The feedstuffs were ground to pass a 3.0-mm mesh screen before incorporation into the diets. Titanium dioxide was included in the diets as indigestible marker. In both experiments, a suspension of pectin (Apple pectin Classic AU 202, Herbstreith and Fox KG, Neuenbuerg, 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 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 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 barrows that did not receive the pectin infusion.

In Exp. 1, the 4 dietary treatments were the control diet, with ileal infusion of water (**Con**–) or pectin (**Con+**); and the control diet supplemented with 15 g/kg of MCP (as-fed basis), together with ileal infusion of water (**MCP**–) or pectin (**MCP+**). The addition of MCP to the control diet at the expense of cornstarch resulted in a dietary P (7.98 g/kg of DM) and Ca (11.49

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

Item	Percentage
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
Soy 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, %	
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 kg 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; and Se, 300 μg.
[2]Calculated according to DLG (1991).

g/kg of DM) level exceeding the P and Ca requirements of growing pigs by approximately 60 and 90%, respectively (DLG, 1991).

In Exp. 2, the 4 dietary treatments included the Con– and Con+ treatments of Exp. 1 and, in addition, the control diet supplemented with 1,000 FTU of phytase/kg (as-fed basis; Natuphos, BASF AG, Ludwigshafen, Germany) together with ileal infusion of water (**Phy**–) or pectin (**Phy+**). Microbial phytase was added to the control diet at the expense of corn starch.

Experimental Procedure

Both experiments were arranged according to a double, incomplete (4 treatments × 2 periods) Latin square design. Each dietary treatment was allotted to 2 of 8 barrows in experimental period I and II, which resulted in 4 observations per treatment. The dietary treatments were randomized among the barrows to ensure that dietary treatments were not administrated twice to the same pig in experimental period I and II. The barrows were fed twice daily, equal amounts 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 corresponds to 1,000 and 1,200 g/d (as-fed basis) in experimental period I and II, respectively. Each experimental period comprised 22 d, including an adaptation period of 15 d. Total collection of feces was initiated at 0700 h on d 16 and ended on d 21 at 0700 h of each experimental period. Feces were collected using 3-L polyethylene bags and a silicon ring, which was attached at the anus region by means of skin adhesive (Medical Adhesive, Hollister, Libertyville, IL) and plaster. The bags were changed each time the feces were voided. After taking subsamples of fresh feces for the analyses of VFA, the remaining feces were stored at –32°C until freeze-drying. Fecal samples for the determination of bacterial enzyme activities were taken by rectal stimulation and subsequently were processed for analysis. Ileal digesta were collected for a total of 24 h in two 12-h intervals: from 0700 to 1900 h on d 21 and from 1900 h on d 22 to 0700 h on d 23. The collection procedure was adapted from that of Li et al. (1993) using plastic tubing attached to the barrel of the cannula by elastic bands. The tubing contained 7 mL of 2.5 *M* formic acid to minimize microbial activity. The tubing was changed a minimum of every 30 min or when it was filled with digesta. Every 2 h, subsamples of ileal digesta of approximately 50 mL were collected for the determination of VFA and lactate. In this case, the tubing used for the digesta collection was devoid of formic acid. Subsamples of digesta for VFA and lactate determination were stored at 4°C until the end of each 12-h digesta collection period. Thereafter, these subsamples were pooled within barrows and stored at –32°C until VFA and lactate analysis. Other samples of ileal digesta were pooled within each animal and period and freeze-dried before analyses.

Isolation of Fecal Mixed Bacterial Mass

After the conclusion of the experiment, fecal samples were thawed, and 1 aliquot was freeze-dried, whereas the second aliquot was used to isolate fecal mixed bacterial mass (**MBM**). The isolation of the MBM was adapted from the procedures described for the isolation of rumen bacteria (Yang et al., 2001). Initially, 500 g of the homogenized fecal sample were suspended in 1,000 mL of a solution containing 0.9% saline and thoroughly stirred in a Waring mixer (Waring Products Division, New Hartford, CT) for 2 min at high speed before being filtered through a double layer of cheesecloth. This procedure was repeated once. Afterwards, the MBM was isolated via differential centrifugation steps. The filtrate was centrifuged at 800 × *g* for 30 min at 4°C (Megafuge 2.0R, Heraeus, Hanau, Germany). The supernatant was immediately transferred into a beaker to avoid renewed mixing of the liquid and solid phase, and stored on ice until the beginning of the next centrifugation step. The second centrifugation step was performed at 27,000 × *g* for 40 min at 4°C (Sorvall RC-5B Refrigerated Superspeed Centrifuge, DuPont Instruments, Bad Nauheim, Germany). The residue was transferred into plastic bottles by means of deionized water and stored at –32°C until

being freeze-dried. After freeze-drying, the MBM was ground to pass through a 0.5-mm mesh screen using a lab ball mill (Mixer Mill MM2000, Retsch, Haan, Germany) before analysis of DM, N, Ca, P, and diaminopimelic acid (**DAP**). In the MBM, total P and Ca concentrations were assayed photometrically by the vanadate-molybdate method (Naumann and Bassler, 1997) and by atomic absorption spectrometry (SpectrAA 220 FS, Varian, Darmstadt, Germany), respectively.

Analytical Methods

Cellulase, amylase, and polygalacturonase activities of fecal bacteria were measured by means of an agar diffusion assay adapted from that of Vahjen et al. (1997; 2005). Agar plates (polycarbonate dishes, 25 × 25 cm, with 170 mL of substrate solution, Nunc, Wiesbaden, Germany) for the determination of cellulase, amylase and polygalacturonase were made of 2.89 g of Gelrite (Roth, Karlsruhe, Germany) and carboxymethylcellulose (**CMC**; 0.5 mg/mL), 2.89 g of Gelrite and starch (5 mg/mL), and 3.4 g of Gelrite and 1.7 g of polygalacturonic acid (**PGA**; 10 mg/mL), respectively. Starch and CMC were dissolved in 5 mL of ethanol and 17 mL of deionized water. These solutions were brought to a volume of 140 mL with 20 m*M* Bis-Tris buffer (pH 6.3) and heated in a microwave at 600 W until boiling. Gelrite was added, and the substrate solutions were homogenized and boiled for 30 min. Finally, the substrate solutions were brought to 170 mL per polycarbonate dish with Bis-Tris buffer.

The hot solutions (approximately 70°C) were poured into the polycarbonate dishes and allowed to harden at room temperature. Polygalacturonic acid was solubilized in 5 mL of ethanol, 17 mL of deionized water, and 17 mL of 2 *N* NaOH and heated. Thereafter, 120 mL of Bis-Tris buffer (Sigma-Aldrich, Steinheim, Germany) was added to the substrate solution with PGA and boiled for 30 min. After cooling to room temperature, the pH was adjusted to 6.3 with 10 *N* HCl. Gelrite was added, the solution was homogenized, and the substrate solution with PGA was boiled for 4 h. Then, the substrate solution was brought to 170 mL with Bis-Tris buffer per polycarbonate dish and poured into the dishes.

Circular wells (6-mm diam.) were punched out of the cold agar plates and filled with either 40 μL of a standard enzyme solution or the fecal sample. Preparation of the fecal samples was carried out as follows: 1 g of fresh fecal sample was diluted at a ratio of 1 to 10 (wt/wt) with deionized water and was homogenized in a shaker for 15 min. The homogenized material was centrifuged at 27,000 × *g* for 20 min at 4°C. The supernatant was filtered under sterile conditions (0.2-μm cellulose-acetate filter) before being transferred to the agar plates. After a 17-h incubation period at 40°C, the agar plates were layered with a solution of 0.2% Congo Red for 25 min. Thereafter, the unbound Congo Red was removed by washing the agar plates with 1 *M* NaCl for 10 min. This step was repeated 3 to 4 times until the Congo Red solution was completely removed. The partial enzymatic degradation of the substrate resulted in lysis zones surrounded by red areas of bound Congo Red. The quantification of enzyme activity on the agar plates was performed by computer assisted evaluation of the lysis zones using digital image capturing and densitometric software PCAS 2.09 (Raytest, Straubenhardt, Germany).

Determination of the standard enzyme activities of polygalacturonase (EC 3.2.1.15; Novo Nordisk A/S, Bagsværd, Denmark), carboxymethylcellulase (EC 3.2.1.89), and β-amylase (EC 2.2.1.2; Megazyme International Ireland Ltd., Wicklow, Ireland), based on the formation of reducing sugars, was performed according to procedures adapted from those of Vahjen et al. (2005). Twenty microliters of the enzyme solution was added to 200 μL of prewarmed (40°C) substrate solution (5 mg/mL, boiled before use) in 20 m*M* Na-acetate buffer (pH 5.0) and incubated at 50°C for 20 min. The reaction was blocked by adding 100 μL of dinitrosalicylic acid solution. After boiling the reaction mixture in a water bath for 5 min, the solution was cooled on ice and diluted with 1 mL of deionized water. The absorbance of the solution was measured at 530 nm. One unit of enzyme activity is defined as the amount of enzyme producing 1 μmol of reducing sugar (as glucose equivalents) per minute at 40°C. The calibration of the dinitrosalicylic acid solution was performed by means of a glucose dilution series according to the methods of Froeck (1999).

Samples of diets, freeze-dried digesta, and feces were finely ground to pass through a 1.0-mm mesh screen (Lab Retsch mill, Haan, Germany). For amino acid analyses, samples were ground to pass through a 0.5-mm mesh screen. Amino acid analyses including DAP were performed using ion-exchange chromatography (Amino Acid Analyzer LC 3000, Eppendorf Biotronic, Maintal, Germany) with postcolumn derivatization with ninhydrin. The content of TiO_2, Ca, and P in diet, ileal digesta, and feces was measured by means of an inductively coupled plasma spectrometer (JY 24 Sequential ICP, Instruments S.A., Grasbrunn, Germany). Ileal samples from day and night collection were pooled per animal and homogenized before VFA and lactate analyses and pH measurement. For analysis of D- and L-lactic acid, a commercially available, photometric test kit (Boehringer, Ingelheim am Rhein, Germany) was used. Concentrations of VFA (acetic, propionic, n-butyric, iso-butyric, n-valeric, and iso-valeric acids) were measured by gas chromatography (HP 6890 Plus GC-System, Hewlett Packard GmbH, Waldbronn, Germany) using 4-methyl-iso-valerianic acid (Fluka, Buchs SG, Switzerland) as the internal standard. The preparation of samples was performed according to the methods of Zijlstra et al. (1977). The pH was determined using a pH meter (Microprocessor pH Meter pH 537, WTW, Weilheim, Germany). Be-

fore pH measurements, fecal samples were diluted 1:10 with deionized water and homogenized, whereas ileal digesta samples were not diluted.

Calculations and Statistical Analysis

Ileal and fecal recovery of P and Ca is defined as the amounts of P and Ca present in the ileal digesta and feces. Recovery of P and Ca was calculated according to Eq. [1]:

$$D_O = A_I \times (I_D/I_I), \quad [1]$$

where D_O is the total output of a nutrient in ileal digesta or feces (g/kg of DMI), A_I is the concentration of a nutrient in ileal digesta or feces (g/kg of DMI), I_D is the marker concentration in the assay diet (g/kg of DM), and I_I is the marker concentration in ileal digesta or feces (g/kg of DM).

Ileal or fecal disappearance of P and Ca are defined as the difference between the dietary intake of P and Ca and the excretion of P and Ca in ileal digesta or feces. Ileal and fecal P and Ca disappearances were calculated by means of Eq. [2]:

$$D_A = A_D - [(I_D \times A_I)/I_I], \quad [2]$$

where D_A is the disappearance of Ca or P from ileal digesta or feces (g/kg of DMI) and A_D is the concentration of Ca and P in the assay diet (g/kg of DM).

Disappearance of P and Ca in the LI was calculated according to Eq. [3]:

$$D_{LI} = D_{feces} - D_{ileal\ digesta}, \quad [3]$$

where D_{LI} is the disappearance of Ca or P in LI (g/kg of DMI), D_{feces} is the disappearance of Ca or P from feces (g/kg of DMI), and $D_{ileal\ digesta}$ is the disappearance of a Ca or P from ileal digesta (g/kg of DMI).

The bacterial contributions of N, P and Ca in feces were estimated as described by Caine et al. (1999), according to Eq. [4], [5], and [6]:

$$\text{Bacterial N/total fecal N} = (DAP_{feces}/DAP_{MBM}) \times (N_{MBM}/N_{feces}) \times 100, \quad [4]$$

$$\text{Bacterial P/total fecal P} = (DAP_{feces}/DAP_{MBM}) \times (P_{MBM}/P_{feces}) \times 100, \text{ and} \quad [5]$$

$$\text{Bacterial Ca/total fecal Ca} = (DAP_{feces}/DAP_{MBM}) \times (Ca_{MBM}/Ca_{feces}) \times 100. \quad [6]$$

The data were analyzed by ANOVA using the MIXED procedure (SAS Inst. Inc., Cary, NC). Fixed effects included animal and treatment. Period and animal within a square were considered as random effects, assuming a compound symmetry variance-covariance structure (type = cs). In Exp. 1, orthogonal contrasts were used to test the effect of the 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 effect of the 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 Kenward-Rogers method (ddfm = kr). A probability level of $P \leq 0.05$ was defined as significant difference, and trends were discussed up to $P < 0.10$.

RESULTS

Barrows recovered well from surgery and remained healthy throughout the experiments. No feed refusal occurred. The average BW of the barrows 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.

Exp. 1

Chemical Composition of MBM. Both MCP addition and ileal infusion of pectin affected the chemical composition of the MBM (Table 2). Supplementation of MCP tended ($P = 0.088$) to reduce the N content in the MBM, whereas pectin infusion tended ($P = 0.054$) to increase it. Supplemental MCP resulted in an increase in the P ($P < 0.001$) and Ca content ($P < 0.004$) of the MBM, whereas the N:P ratio was decreased ($P < 0.001$) by MCP addition. There was an interaction ($P = 0.023$) of the MCP diet and pectin infusion on the N:P ratio according to which pectin infusion decreased the N:P ratio in the MBM of barrows fed the control diet, but increased the N:P ratio when MCP was added to the diet. Moreover, the ileal infusion of pectin tended ($P = 0.081$) to reduce the Ca content of the MBM.

Microbial Activity. The infusion of pectin into the distal ileum increased ($P = 0.032$) bacterial polygalacturonase activity, whereas bacterial cellulase and amylase activities were not affected (Table 3). Ileal pH was not affected by any of the treatments, whereas pectin infusion decreased ($P = 0.048$) fecal pH. Supplemental MCP reduced ($P = 0.014$) ileal D+L-lactate concentrations, whereas the infusion of pectin decreased ($P = 0.049$) ileal total VFA concentrations (Table 4). Furthermore, the infusion of pectin tended ($P = 0.079$) to increase fecal total VFA concentrations. Ileal DAP concentrations were decreased ($P = 0.029$) following the infusion of pectin. The contribution of bacterial N to total N in feces was similar between treatments ranging from 64 to 70%. There was a main effect of MCP on the contribution of bacterial P and Ca to total P and Ca in feces. In fact, supplemental MCP increased ($P = 0.021$) the contribution of bacterial P to total P in feces by 24.4 and 14.8 percentage units in the MCP– and MCP+ compared with the Con– and Con+ treatment, respec-

Table 2. Effects of dietary monocalcium phosphate (MCP) supplementation and ileal pectin infusion on chemical composition of fecal mixed bacterial mass (Exp. 1)

	Treatment[1]							
	Control diet		MCP diet			Contrast, *P*-value		
Item	Water infusion[2]	Pectin infusion[3]	Water infusion[2]	Pectin infusion[3]	SEM	Pectin vs. water infusion	MCP vs. control diet	Pectin infusion × MCP diet
N, g/kg of DM	56.3	59.1	51.5	57.7	1.50	0.054	0.088	0.304
P, g/kg of DM	22.2	21.7	36.9	32.7	1.27	0.185	<0.001	0.192
N:P ratio, g/g	2.73	2.65	1.47	1.91	0.08	0.126	<0.001	0.023
Ca, g/kg of DM	27.9	21.9	49.0	36.6	3.57	0.081	0.004	0.394
Ca:P ratio, g/g	1.15	1.07	1.32	1.09	0.07	0.133	0.243	0.334
DAP,[4] g/kg of DM	2.0	2.3	1.6	2.2	0.22	0.139	0.274	0.741

[1]Values are least squares means of 4 observations per treatment.
[2]A total of 1.8 L of demineralized water were infused per day.
[3]A total of 60 g/d of pectin were suspended in 1.8 L of demineralized water at a ratio of 1:30 (wt/wt).
[4]DAP = diaminopimelic acid.

tively. Similarly, the contribution of bacterial Ca to total Ca in feces was increased ($P = 0.030$) by 13.8 and 9.7 percentage units in the MCP– and MCP+ compared with the Con– and Con+ treatment, respectively.

P and Ca Disappearance and Recovery. The supplementation of MCP resulted in greater P and Ca intakes of 7.25 and 11.49 g/kg of DMI compared with the control treatments with 3.25 and 7.98 g/kg of DMI, respectively (Table 5). Ileal and total tract P disappearance ($P < 0.001$) as well as ileal and fecal P recoveries increased ($P \leq 0.003$) in response to MCP addition (Table 5), and the net P secretion into the LI tended ($P = 0.073$) to be greater following MCP supplementation. Ileal and total tract Ca disappearance as well as ileal and fecal Ca recovery was also increased ($P \leq 0.010$) by MCP addition. Moreover, there was an interaction ($P = 0.034$) between MCP diet and ileal pectin infusion on ileal Ca disappearance. In barrows fed the control diet, pectin infusion decreased the ileal Ca disappearance, whereas it caused a greater ileal Ca disappearance when MCP was added to the diet.

Exp. 2

Chemical Composition of MBM. Both supplementation of phytase to the control diet and ileal infusion of pectin affected the chemical composition of the MBM (Table 6). Phytase supplementation tended ($P = 0.094$) to reduce the N content and decreased ($P = 0.045$) the P content of the MBM, but tended ($P = 0.053$) to increase the N:P ratio. Both pectin infusion ($P = 0.033$) and phytase supplementation ($P = 0.021$) increased the Ca:P ratio, whereas pectin infusion tended ($P = 0.069$) to increase the DAP concentration of the MBM.

Microbial Activity. Bacterial cellulase activity in feces tended ($P = 0.089$) to decrease in response to phytase supplementation, and the infusion of pectin reduced it ($P = 0.036$; Table 7). Bacterial polygalacturonase activity, in turn, increased ($P < 0.001$) in response to the ileal pectin infusion. Supplemental phytase or pectin infusion did not affect ileal or fecal total VFA, ileal lactate concentrations, or ileal pH (Table 8). However, supplemental phytase increased ($P = 0.008$) fecal pH. The infusion of pectin tended ($P = 0.099$) to increase the DAP concentrations in feces. The contribution of bacterial N and P in the MBM in relation to the total amount of N and P in feces, respectively, was similar between the treatments. However, the contribution of bacterial Ca to total Ca in feces was increased ($P = 0.016$) by pectin infusion and tended ($P = 0.061$) to be enhanced by phytase supplementation.

Table 3. Effects of dietary monocalcium phosphate (MCP) supplementation and ileal pectin infusion on bacterial carbohydrase activity (IU/kg of DM) in feces (Exp. 1)

	Treatment[1]							
	Control diet		MCP diet			Contrast, *P*-value		
Item	Water infusion[2]	Pectin infusion[3]	Water infusion[2]	Pectin infusion[3]	SEM	Pectin vs. water infusion	MCP vs. control diet	Pectin infusion × MCP diet
Cellulase	0.01	0.01	0.01	0.01	0.001	0.919	0.427	0.909
Amylase	2.37	2.26	2.25	1.87	0.581	0.738	0.672	0.820
Polygalacturonase	5.48	27.36	5.19	30.37	6.734	0.032	0.843	0.826

[1]Values are least squares means of 4 observations per treatment.
[2]A total of 1.8 L of demineralized water were infused per day.
[3]A total of 60 g/d of pectin were suspended in 1.8 L of demineralized water at a ratio of 1:30 (wt/wt).

Table 4. Effects of dietary monocalcium phosphate (MCP) supplementation and ileal pectin infusion on ileal and fecal pH, VFA, lactate, and diaminopimelic acid concentrations (Exp. 1)

	Treatment[1]					Contrast, *P*-value		
	Control diet		MCP diet					
Item	Water infusion[2]	Pectin infusion[3]	Water infusion[2]	Pectin infusion[3]	SEM	Pectin vs. water infusion	MCP vs. control diet	Pectin infusion × MCP diet
Ileal pH	6.8	6.6	6.9	6.8	0.09	0.337	0.221	0.767
Fecal pH	7.0	6.5	7.3	6.8	0.15	0.048	0.128	0.956
Ileal D+L-lactate, mg/g of DM	37.8	32.3	15.3	27.0	3.33	0.504	0.014	0.387
Ileal total VFA,[4] mmol/kg of DM	339	335	508	291	31.60	0.049	0.122	0.399
Fecal total VFA,[4] mmol/kg of DM	279	398	274	388	44.78	0.079	0.872	0.705
Ileal DAP,[5] mg/g of DM	0.14	0.14	0.20	0.11	0.01	0.029	0.199	0.315
Fecal DAP,[5] mg/g of DM	0.93	1.04	0.82	1.01	0.06	0.120	0.326	0.811
MBM-P:fecal P ratio,[6] %	44.4	52.8	68.8	67.6	7.37	0.632	0.021	0.545
MBM-Ca:fecal P ratio,[6] %	40.8	35.5	54.6	45.2	4.04	0.188	0.030	0.623
MBM-N:fecal N ratio,[6] %	64.9	64.0	70.3	69.0	4.68	0.852	0.318	0.969

[1]Values are least squares means of 4 observations per treatment.
[2]A total of 1.8 L of demineralized water were infused per day.
[3]A total of 60 g/d of pectin were suspended in 1.8 L of demineralized water at a ratio of 1:30 (wt/wt).
[4]Total VFA = sum of acetic, propionic, n-butyric, iso-butyric, n-valeric, and iso-valeric acid.
[5]DAP = diaminopimelic acid.
[6]MBM = mixed bacterial mass.

P and Ca Disappearance and Recovery. Supplemental phytase substantially increased ($P < 0.001$) ileal and total tract P disappearance and therefore reduced ($P < 0.001$) ileal and fecal P recoveries (Table 9). Ileal and total tract Ca disappearance and recovery were not affected by phytase or pectin. However, there was an interaction of phytase diet and pectin infusion on the ileal Ca disappearance ($P = 0.055$) and recovery ($P = 0.048$). In barrows fed the control diet, pectin infusion increased the ileal Ca disappearance, whereas in barrows fed the MCP diet pectin infusion decreased the ileal Ca disappearance. In contrast, the ileal Ca recovery was decreased by infusing pectin in barrows fed the control diet, whereas pectin increased the ileal Ca recovery when MCP was added to the diet.

DISCUSSION

The results of the present study confirm the original hypothesis that microbial induced incorporation of P into the MBM isolated from pig's feces may depend on the dietary P supply. Similar to P, the bacterial Ca assimilation also responded to differences in dietary Ca supply. In fact, the addition of MCP to the diet in Exp.

Table 5. Effects of dietary monocalcium phosphate (MCP) supplementation and ileal pectin infusion on ileal and fecal phosphorus and calcium disappearance and recovery (Exp. 1)

	Treatment[1]					Contrast, *P*-value		
	Control diet		MCP diet					
Item	Water infusion[2]	Pectin infusion[3]	Water infusion[2]	Pectin infusion[3]	SEM	Pectin vs. water infusion	MCP vs. control diet	Pectin infusion × MCP diet
P intake, g/kg of DM	3.25	3.25	7.25	7.25				
Ileal P disappearance, g/kg of DMI	0.85	0.97	3.67	3.90	0.138	0.337	<0.001	0.683
Ileal P recovery, g/kg of DMI	2.41	2.27	3.57	3.36	0.106	0.222	<0.001	0.781
Total tract disappearance of P, g/kg of DMI	0.82	0.95	3.63	3.35	0.295	0.847	<0.001	0.507
Fecal P recovery, g/kg of DMI	2.44	2.14	3.61	3.56	0.25	0.587	0.003	0.628
P disappearance in LI,[4] g/kg of DMI	–0.02	0.04	–0.13	–0.52	0.154	0.307	0.073	0.278
Ca intake (g/kg of DM)	7.98	7.98	11.49	11.49				
Ileal Ca disappearance, g/kg of DMI	4.28	4.07	5.32	6.03	0.187	0.216	<0.001	0.034
Ileal Ca recovery, g/kg of DMI	3.76	3.84	6.23	5.52	0.284	0.389	<0.001	0.214
Total tract disappearance of Ca, g/kg of DMI	4.22	4.13	5.58	5.46	0.335	0.811	0.010	0.970
Fecal Ca recovery, g/kg of DMI	3.82	3.43	5.84	5.54	0.531	0.608	0.010	0.938
Ca disappearance in LI, g/kg of DMI	–0.29	0.11	0.29	–0.28	0.293	0.772	0.779	0.251

[1]Values are least squares means of 4 observations per treatment.
[2]A total of 1.8 L of demineralized water were infused per day.
[3]A total of 60 g/d of pectin were suspended in 1.8 L of demineralized water at a ratio of 1:30 (wt/wt).
[4]LI = large intestine.

Table 6. Effects of dietary phytase supplementation and ileal pectin infusion on chemical composition of fecal mixed bacterial mass (Exp. 2)

	Treatment[1]							
	Control diet		Phytase diet			Contrast, *P*-value		
Item	Water infusion[2]	Pectin infusion[3]	Water infusion[2]	Pectin infusion[3]	SEM	Pectin vs. water infusion	Phytase vs. control diet	Pectin infusion × phytase diet
N, g/kg of DM	58.5	56.2	55.0	52.9	1.69	0.327	0.094	0.925
P, g/kg of DM	22.0	17.3	13.5	12.2	2.61	0.381	0.045	0.524
N:P ratio, g/g	2.84	3.48	3.91	4.42	0.41	0.287	0.053	0.880
Ca, g/kg of DM	22.9	22.3	17.0	19.6	3.58	0.822	0.267	0.658
Ca:P ratio, g/g	0.99	1.30	1.28	1.53	0.08	0.033	0.021	0.686
DAP,[4] g/kg of DM[4]	2.0	2.6	2.1	2.4	0.23	0.069	0.969	0.867

[1]Values are least squares means of 4 observations per treatment.
[2]A total of 1.8 L of demineralized water were infused per day.
[3]A total of 60 g/d of pectin were suspended in 1.8 L of demineralized water at a ratio of 1:30 (wt/wt).
[4]DAP = diaminopimelic acid.

1 increased the P content in the MBM by approximately 66 and 51% and the Ca content by 76 and 67% in the MCP– and MCP+ treatment compared with Con– and Con+ treatment, respectively. These results are in general agreement with those obtained in rats. After being fed graded levels of Ca-phosphate, an increased fecal excretion of phospholipids, nitrogen and organic phosphate of bacterial origin was observed (Bovee-Oudenhoven et al., 1997). Moreover, in the present study MCP supplemented to a P-deficient diet increased the proportion of bacterial P to total fecal P, indicating that the bacterial assimilation of P was stimulated due to greater concentrations of P available in the GIT. In fact, under the conditions of an excessive dietary P supply, intestinal bacteria have the potential to store P in the form of polyphosphates (Wood and Clark, 1988). Similarly, supplemental MCP increased the proportion of bacterial Ca to total Ca in feces. Because polyphosphates are negatively charged, the increased bacterial assimilation of Ca is likely related to the neutralization of polyphosphates. It is generally known that several cations, such as Ca, Mg, and K, play an important role in the neutralization and stabilization of polyphosphate ions in bacterial cells (e.g., Buchan, 1983; Van Groenestijn et al., 1988).

Supplemental MCP stimulated bacterial incorporation of P and Ca but not of N in MBM. In contrast, with the ileal infusion of pectin, an increase in bacterial N assimilation was observed in Exp. 1. Obviously, bacterial N assimilation in the LI was limited due to a shortage in fermentable substrate such as pectin which is in accordance with observations in growing-finishing pigs when starch was infused into the ileum (Mosenthin et al., 1992). In addition, the present observation confirms results of Bovee-Oudenhoven et al. (1997) obtained in studies with rats. These authors showed a substantial increase in the fecal excretion of bacterial N when a diet containing both fermentable substrate in the form of lactulose and supplemental Ca-phosphate (approximately 80% above the requirement of laboratory rats, NRC, 1995) was fed. However, this response was much less pronounced when the diet was supplemented with Ca-phosphate only.

In the present study, there was no increase in the bacterial cellulase activity in feces in response to supplemental MCP as observed by Francis et al. (1978) under in vitro conditions. According to these authors, rumen cellulase activity was greater when the concentration of phosphate was increased from 5 to 50 m*M*, and increasing Ca concentrations had no effect on cellu-

Table 7. Effects of dietary phytase supplementation and ileal pectin infusion on bacterial carbohydrase activity (IU/g of DM) in feces (Exp. 2)

	Treatment[1]							
	Control diet		Phytase diet			Contrast, *P*-value		
Item	Water infusion[2]	Pectin infusion[3]	Water infusion[2]	Pectin infusion[3]	SEM	Pectin vs. water infusion	Phytase vs. control diet	Pectin infusion × phytase diet
Cellulase	0.06	0.02	0.03	0.01	0.008	0.036	0.089	0.827
Amylase	12.65	4.50	11.38	4.95	3.843	0.171	0.918	0.133
Polygalacturonase	5.53	29.67	3.75	37.07	3.253	<0.001	0.414	0.206

[1]Values are least squares means of 4 observations per treatment.
[2]A total of 1.8 L of demineralized water were infused per day.
[3]A total of 60 g/d of pectin were 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 ileal and fecal pH values, VFA, lactate and diaminopimelic acid concentrations (Exp. 2)

	Treatment[1]					Contrast, *P*-value		
	Control diet		Phytase diet					
Item	Water infusion[2]	Pectin infusion[3]	Water infusion[2]	Pectin infusion[3]	SEM	Pectin vs. water infusion	Phytase vs. control diet	Pectin infusion × phytase diet
Ileal pH	6.7	6.7	6.8	6.8	0.12	0.931	0.328	0.955
Fecal pH	6.9	6.9	7.2	7.1	0.07	0.906	0.008	0.961
Ileal D+L-lactate, mg/g of DM	48.6	28.1	36.8	35.1	6.91	0.234	0.733	0.220
Ileal total VFA,[4] mmol/kg of DM	294	295	355	262	32.64	0.289	0.677	0.197
Fecal total VFA,[4] mmol/kg of DM	331	339	295	277	24.46	0.870	0.104	0.714
Ileal DAP,[5] mg/g of DM	0.17	0.20	0.25	0.19	0.03	0.606	0.140	0.076
Fecal DAP,[5] mg/g of DM	0.99	1.13	1.01	1.23	0.10	0.099	0.615	0.917
MBM-P:fecal P ratio,[6] %	57.0	43.2	56.1	61.2	5.77	0.468	0.162	0.598
MBM-Ca:fecal Ca ratio,[6] %	29.1	39.6	35.1	44.5	2.24	0.016	0.061	0.801
MBM-N:fecal N ratio,[6] %	68.6	69.6	69.2	72.2	3.63	0.668	0.661	0.794

[1]Values are least squares means of 4 observations per treatment.
[2]A total of 1.8 L of demineralized water were infused per day.
[3]A total of 60 g/d of pectin were suspended in 1.8 L of demineralized water at a ratio of 1:30 (wt/wt).
[4]Total VFA = sum of acetic, propionic, n-butyric, iso-butyric, n-valeric, and iso-valeric acid.
[5]DAP = diaminopimelic acid.
[6]MBM = mixed bacterial mass.

lase activity. Apparently, in the present study the P concentration of the control diet was sufficient to maintain the cellulolytic activity in the pig's LI so that supplemental MCP had no further effect. Moreover, increasing of P supply enhanced production of VFA and ATP of rumen bacteria in vitro (Komisarczuk et al., 1987b). In the present study, supplemental MCP did not significantly increase ileal or fecal VFA concentration. However, according to Cummings (1981) 95% of the VFA generated in the GIT are absorbed during transit of digesta through the gut. Thus, measurement of VFA in ileal digesta and feces may not necessarily reflect the total amount of VFA produced in the GIT. However, the significantly reduced ileal D+L-lactate concentrations when MCP was supplemented may indicate changes in the population of lactic acid producing bacteria, such as lactobacilli, enterococci, and bifidobacteria, their metabolic activity, or both (Ewing and Cole, 1994).

The reduction in P recovery in ileal digesta due to phytase supplementation (Exp. 2) decreased the amount of P available for bacterial utilization in the LI as reflected by decreased P contents in the MBM of the Phy– and Phy+ compared with the Con– and Con+ treatments. These values correspond well with those

Table 9. Effects of dietary phytase supplementation and ileal pectin infusion on ileal and fecal phosphorus and calcium disappearance and recovery in pigs (Exp. 2)

	Treatment[1]					Contrast, *P*-value		
	Control diet		Phytase diet					
Item	Water infusion[2]	Pectin infusion[3]	Water infusion[2]	Pectin infusion[3]	SEM	Pectin vs. water infusion	Phytase vs. control diet	Pectin infusion × phytase diet
P intake, g/kg of DM	3.01	3.01	3.06	3.06				
Ileal P disappearance, g/kg of DMI	0.95	0.92	1.89	1.92	0.089	0.948	<0.001	0.753
Ileal P recovery, g/kg of DMI	2.10	2.06	1.20	1.09	0.073	0.427	<0.001	0.670
Total tract disappearance of P, g/kg of DMI	0.90	0.96	1.75	1.74	0.083	0.827	<0.001	0.680
Fecal P recovery, g/kg of DMI	2.10	1.93	1.31	1.20	0.098	0.269	<0.001	0.740
P disappearance in LI,[4] g/kg of DMI	0.00	–0.01	–0.11	–0.20	0.917	0.644	0.139	0.562
Ca intake, g/kg of DM	8.89	8.89	8.39	8.39				
Ileal Ca disappearance, g/kg of DMI	5.46	6.23	5.83	5.29	0.272	0.731	0.329	0.055
Ileal Ca recovery, g/kg of DMI	3.36	2.73	2.63	3.03	0.202	0.645	0.323	0.048
Total tract disappearance of Ca, g/kg of DMI	5.12	5.27	5.86	5.40	0.405	0.760	0.318	0.472
Fecal Ca recovery, g/kg of DMI	3.69	3.29	2.59	2.54	0.489	0.705	0.109	0.725
Ca disappearance in LI, g/kg of DMI	–0.13	–0.58	0.25	–0.69	0.399	0.105	0.781	0.580

[1]Values are least squares means of 4 observations per treatment.
[2]A total of 1.8 L of demineralized water were infused per day.
[3]A total of 60 g/d of pectin were suspended in 1.8 L of demineralized water at a ratio of 1:30 (wt/wt).
[4]LI = large intestine.

obtained in rumen bacterial mass in which in response to a decline in P concentrations of rumen fluid, a decrease in P concentrations from 14.7 to 6.1 g/kg (DM) was observed (Komisarczuk et al., 1987b). In contrast, ileal and fecal recovery and disappearance of Ca as well as the bacterial Ca assimilation into the MBM were not affected by phytase supplementation. Nevertheless, the bacterial proportion of Ca to total Ca in feces tended to increase when phytase was supplemented.

In ruminants, P depletion reduced the fermentative activity of rumen bacteria resulting in decreased production of VFA and bacterial ATP (Komisarczuk et al., 1987b). In the present study, phytase did not significantly affect VFA concentrations in feces. However, the positive effect of pectin on fecal VFA concentrations could not be repeated, which might be an indication that (comparable with rumen bacteria) the P availability in the LI might have limited bacterial degradative activity. The higher fecal pH may support decreased VFA production in the LI of pigs fed the phytase containing diet. In addition, phytase supplementation tended to reduce the fecal bacterial cellulase activity, which may be also attributed to the lower P availability in the LI, whereas the bacterial polygalacturonase and amylase activities were not affected. Accordingly, data from in vitro studies with rumen bacteria confirm that the cellulolytic activity was much more affected by P deficiency than the activity of other carbohydrases (Durand and Komisarczuk, 1988). Moreover, growth and cellulase activity of *Bacteroides succinogenes,* a major rumen bacterium that also occurs in the LI of pigs (Varel et al., 1984), was suppressed when cultured in a P-deficient medium (Komisarczuk et al., 1988). Apparently, the decreased supply of available P to large intestinal bacteria due to phytase supplementation reduced the fermentation intensity in the LI similarly to rumen bacteria. Moreover, bacterial protein synthesis and growth yield of rumen bacteria were largely reduced under P-deficient conditions (Komisarczuk et al., 1987a), which is in agreement with the trend toward decreased bacterial N assimilation obtained for the Phy– and Phy+ treatments.

There were several treatment effects on the N:P ratio in both experiments and the Ca:P ratio of the MBM in Exp. 2. Due to greater intestinal P availability for the MCP– and MCP+ treatments, a lower N:P ratio was obtained, whereas a reduction in intestinal P supply due to phytase supplementation resulted in a greater N:P ratio. Similarly, the Ca:P ratio of the MBM was increased considerably by the ileal infusion of pectin and phytase supplementation in Exp. 2, but in Exp. 1 the pectin infusion had no effect on the Ca:P ratio of the MBM. It is unknown if these differences between experiments may be attributed to variations in the bacterial community among individual pigs. For example, the bacterial community inhabiting the GIT is strongly homeostatic and characteristic for the host from which the digesta sample was obtained (Zoetendal et al., 1998; Hill et al., 2005).

In contrast to the differences in dietary P supply, the ileal infusion of pectin did not affect the P contents of the MBM in the present study, although there is evidence that pectin may affect the composition and activity of the microflora in the LI of rats and pigs (Dongowski et al., 2002; Owusu-Asiedu et al., 2006). Moreover, a study in pigs revealed that the oral administration of a diet containing 300 g of pectin/kg decreased the P content of the MBM from 23.3 to 13.2 g/kg of DM compared with the control diet without pectin (Metzler et al., 2006). It has to be emphasized, however, that in the aforementioned study approximately 300 g of pectin were fed daily, whereas in this study only 60 g of pectin were supplied daily via the ileal cannula. Obviously, the degree of P incorporation into the MBM is strongly dependant on the supply of fermentable substrates such as pectin.

As pectin was infused into the distal ileum, it was expected that the main pectin effects on bacterial activity could be observed in the LI. However, bacterial flow and activity were already affected in the distal ileum as suggested by decreased ileal VFA and DAP concentrations. According to previous reports, pectin is almost completely fermented by the colonic microflora (Dongowski et al., 2000, 2002). This is confirmed by the trends of greater fecal VFA concentrations and significantly lower pH in feces after ileal pectin application in Exp. 1. but not in Exp 2. Because Con– and Con+ treatments did not differ between both experiments, differences in the level of microbial metabolites may be attributed to variations in the microbial composition and metabolic activity in the hindgut among individual pigs. Moreover, in the present study, ileal infusion of pectin largely stimulated the pectinolytic activity determined at the fecal level, likely reflecting changes in the composition of the microflora. For example, Dongowski et al. (2002) reported an increase in the populations of total anaerobic bacteria and *Bacteroides* spp. in feces of rats fed 6.5% of pectin for 3 wk. Generally, pectin is degraded by different intestinal bacterial groups including *Bacteroides* spp., clostridia, and bifidobacteria (Dongowski et al., 2000; Olano-Martin et al., 2002). Furthermore, changes in the bacterial metabolic activity and species composition are reflected by a reduction in fecal bacterial cellulolytic activity following the infusion of pectin in Exp. 2. However, this effect was not observed in Exp. 1, probably due to individual variations among pigs used in both experiments. In addition, the contribution of bacterial Ca to total Ca in feces was raised by pectin infusion in Exp. 2 but not in Exp. 1, likely due to changes in bacterial metabolic activity.

Although pectin did not affect fecal total VFA concentrations in Exp. 2, the trend of greater DAP concentrations in feces following pectin infusion may indicate stimulation of bacterial growth and activity. This is supported by Mosenthin et al. (1994) who observed a greater fecal output of bacterial N in pigs fed with diets containing 7.5% of pectin compared with the control pigs. Although DAP is only found in bacterial cell wall

mucoproteins (Rowan et al., 1992), it has to be considered that DAP concentrations may vary considerably among different bacterial species. For example, some gram-positive bacteria are completely devoid of DAP (Dufva et al., 1982).

In conclusion, the present data clearly show that the bacterial P and Ca incorporation and fermentation in pigs is affected by both dietary P and Ca level and the availability of fermentable substrate such as pectin. Increasing the dietary P and Ca level by supplemental MCP increased the bacterial incorporation of P and Ca into the MBM due to greater bacterial intestinal P availability. In contrast, reducing the intestinal P availability through phytase supplementation to low-P diets reduced bacterial P incorporation into the MBM and lowered fermentation activity. Further research is warranted to clarify some of the inconsistency in the results between both experiments to better predict the relationships between dietary P and Ca supply, the availability of fermentable substrate and the microbial fermentation in the LI of pigs.

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