Bacterial fermentation affects net mineral flux in the large intestine of pigs fed diets with viscous and fermentable nonstarch polysaccharides

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Bacterial fermentation affects net mineral flux in the large intestine of pigs fed diets with viscous and fermentable nonstarch polysaccharides[1,2]

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ABSTRACT: The impact of colonic fermentation on postileal absorption of Ca, Mg, P, Cu, Fe, Mn, and Zn was investigated in 8 ileally cannulated grower pigs (initial BW = 29.1 ± 1.6 kg) according to a double 4 × 4 Latin square. A semi-purified diet was supplemented with 5.20% low viscous, low fermentable cellulose (CEL), 6.25% high viscous, low fermentable carboxymethylcellulose (CMC), 8.95% low viscous, high fermentable oat β-glucan (LG), or 9.25% high viscous, high fermentable oat β-glucan (HG), resulting in 5% actual added nonstarch polysaccharides (NSP) in the diets. Because of the intrinsic mineral content in LG and HG, pigs receiving the LG and HG diets had a greater ($P < 0.05$) daily intake of Mg, P, Cu, Fe, Mn, and Zn, and also Ca for the HG diet compared with the CEL and CMC diets. Different amounts of minerals reached the large intestine for the 4 diets as indicated by the 60 to 86% less ($P < 0.05$) ileal flow of Ca, Mg, P, and Fe for CMC compared with CEL and HG. Apparent mineral retention was generally less ($P < 0.05$) for CEL compared with CMC. Regression analyses indicated that postileal flux of Ca, Cu, and Zn were related ($R^2 = 0.24$ to 0.99; $P < 0.05$) to short-chain fatty acid (SCFA) concentrations in feces. Postileal Ca absorption was negatively related ($R^2 = 0.24$; $P < 0.05$) to fecal total SCFA concentrations when SCFA concentrations were greater than 95.6 mmol/kg of DM. Furthermore, postileal Zn ($R^2 = 0.99$; $P < 0.001$) and Cu secretion ($R^2 = 0.94$; $P < 0.001$) decreased with increasing total SCFA concentrations in feces. Additionally, postileal secretion of Fe increased ($R^2 = 0.20$; $P < 0.05$) with increasing 16S rRNA gene copies of *Enterobacteriaceae* in feces, whereas the secretion of Cu decreased ($R^2 = 0.25$; $P < 0.01$) with increasing gene copies of *Enterobacteriaceae*. Overall, the apparent retention of Ca, Mg, and P was 27 to 85% less ($P < 0.05$) for CEL and HG than for CMC, whereas the apparent retention of Fe, Mn, and Zn was less ($P < 0.05$) for CEL than for CMC, LG, and HG. In conclusion, these data indicate that the stimulation of fermentation by dietary NSP affects net mineral flux in the large intestine that, in turn, can influence mineral excretion in feces. Additionally, negative effects of CEL on apparent retention may increase the daily requirement for minerals of grower pigs.

Key words: bacteria, fermentation, mineral, nonstarch polysaccharide, pig

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INTRODUCTION

Coproducts containing nonstarch polysaccharide (**NSP**) fractions are increasingly available from the bioprocessing industry for use in pig feeds and are attractive economically. Aside from phytic acid, functional properties of NSP, such as viscosity, solubility, and water- and ion-binding capacity, may impair mineral absorption in the gastrointestinal tract (**GIT**) of pigs (Idouraine et al., 1996; Dikeman and Fahey, 2006; Metzler and Mosenthin, 2008). Thus, a need exists to define effects of NSP fractions, such as cellulose and β-glucans, on mineral absorption in the small intestine and to elucidate the consequences of fermentation of complex NSP on mineral absorption in the large intestine of pigs.

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[2]A portion of this study was presented at the Digestive Physiology in Pigs conference in Montbrió del Camp, Spain (May 20 to 22, 2009), and will be included as a short communication in the conference proceedings.

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In humans and rats, supplementation of fructans and resistant starch stimulated colonic Ca and Mg absorption associated with short-chain fatty acid (**SCFA**) production in the lower GIT (Abrams et al., 2007; Demigné et al., 2008). Additionally, bacteria may compete with the host for minerals by incorporating substantial amounts to meet their own mineral requirements for fermentation and growth, thereby likely decreasing available minerals for the host (Hrdina et al., 2009; Metzler et al., 2009). Therefore, the contribution of the colon in the overall absorption of minerals is important to consider, even though the small intestine is recognized as the major site of mineral absorption in pigs (Schröder and Breves, 2006). Thus, we hypothesized that bacterial fermentation and growth, as influenced by the availability of different NSP, may affect colonic mineral absorption in pigs.

The aim of the present study was to investigate the relation among fecal SCFA, fecal numbers of bacterial groups, and postileal net mineral flux in pigs fed diets with complex NSP using regression analysis. Moreover, the effects of purified NSP fractions of different functional properties (i.e., viscosity and fermentability) on ileal flow and apparent retention of minerals were assessed.

MATERIALS AND METHODS

The animal protocol was approved by the University of Alberta Animal Care and Use Committee for Livestock and followed principles established by the Canadian Council on Animal Care (1993).

Animals and Diets

Eight crossbred barrows (Duroc × Large White) were surgically fitted with a T-cannula at the distal ileum (Li et al., 1993) at an average BW of 22.2 ± 1.4 kg. Pigs were housed in individual metabolism pens (1.2 × 1.2 m), with a plastic-coated, expanded metal floor, polyvinyl chloride walls (0.9 m high), and plexiglass windows (0.3 × 0.3 m), allowing freedom of movement and visual contact to other pigs. Pigs had free access to drinking water.

A semipurified diet (Table 1) consisting of cornstarch and casein was formulated to meet or exceed the trace mineral requirements for grower pigs on a gram per kilogram basis (NRC, 1998) so that a dietary content below swine trace mineral requirements would not interfere with observations for interactions between dietary NSP and trace minerals (Idouraine et al., 1995, 1996). The dietary content of Ca, Mg, and P was formulated to be marginally deficient to not affect their absorption because of homeostatic regulatory mechanisms. Otherwise, with increasing dietary content of these minerals, increasing amounts of minerals will be excluded from absorption (Rodehutscord et al., 1999; Zimmermann et al., 2002). Moreover, a dietary Ca content above the requirement may interfere with trace mineral absorption (Hallberg et al., 1991; Krebs, 2000). The basal diet was supplemented with 4 NSP fractions: 1) low viscous, low fermentable microcrystalline cellulose (**CEL**; TIC Pretested Ticacel 100-S, TIC GUMS, White Marsh, MD), 2) high viscous, low fermentable sodium carboxymethylcellulose (**CMC**; TIC Pretested Ticalose CMC 6000 F, TIC GUMS; Na, 7.943 g/kg), 3) low viscous, high fermentable oat β-glucan (**LG**; OatVantage, GTC Nutrition, Missoula, MT), and 4) high viscous, high fermentable oat β-glucan (**HG**; Viscofiber, Cevena Bioproducts, Edmonton, Alberta, Canada), respectively. The NSP fractions were selected with respect to their in vitro viscosity that was 0.3, 285, 20, and 210 mPa·s for CEL, CMC, LG, and HG, respectively, as determined in 0.5% NSP solution using a rheometer (UDS 200, Paar Physica, Glenn, VA) at a shear rate of 12.9 s^{-1} and 20°C. The LG and HG were β-glucan concentrates because the wet fractioning process from the feedstock oat did not allow 100% purification to ensure that the functional properties of β-glucan are maintained (Vasanthan and Temelli, 2008). A β-glucan concentrate also contains residues of other fiber, starch, protein, lipid, and ash beside the actual β-glucan (Hooda et al., 2010a). To reach 5% of the actual NSP in the diet, the inclusion percentages of the NSP fractions were 5.20, 6.25, 8.95, and 9.25% for CEL, CMC, LG, and HG, respectively. The NSP fractions were added at the expense of cornstarch. The amounts of Ca, Mg, P, Cu, Fe, Mn, and Zn originating from the non-NSP part of the diet were similar among diets. The calculated and analyzed amounts of Fe and Mn differed in the basal diet; therefore, the inorganic Ca and P sources (i.e., limestone and dicalcium phosphate) were analyzed. Dicalcium phosphate supplied 113 and 5 mg/kg of diet of Fe and Mn, respectively. Titanium dioxide was included as indigestible marker. Pigs were allowed to consume the experimental diets at 3× their maintenance requirement for energy (3 × 110 kcal of DE/kg of $BW^{0.75}$; NRC, 1998) in 2 equal meals at 0800 and 1600 h.

Experimental Design

After a 10-d recovery, pigs were fed the experimental diets according to a double 4 × 4 Latin square. Each of the diets was allotted to 2 out of 8 pigs per period, resulting in 8 observations per diet. Each experimental period was composed of 17 d, including an adaptation of 10 d to the diets followed by a 3-d total collection of feces and urine and a 4-d collection of ileal digesta. Feces were collected using plastic bags attached to the skin around the anus (van Kleef et al., 1994). Urine was collected in boxes containing 60 mL of concentrated sulfuric acid and stored at −20°C. Ileal digesta were collected from 0800 to 1600 h on d 14, 15, 16, and 17 using plastic tubing attached to the barrel of the cannula by elastic bands (Li et al., 1993). The tubing contained 15 mL of formic acid (5%) to minimize further microbial fermentation. Thereafter, feces and digesta were pooled

Table 1. Ingredient and analyzed chemical composition of diet[1]

Item	Low fermentable		High fermentable	
	Low viscous CEL	High viscous CMC	LG	HG
Ingredient, g/kg (as-fed basis)				
Corn starch	729.0	718.5	688.5	674.6
Casein	160.0	160.0	160.0	160.0
CEL	52.0	—	—	—
CMC	—	62.5	—	—
LG	—	—	89.5	—
HG	—	—	—	92.5
Dicalcium phosphate[2]	12.0	12.0	12.0	12.0
Celite	10.0	10.0	10.0	10.0
Canola oil	10.0	10.0	10.0	10.0
Limestone	9.0	9.0	9.0	9.0
Mineral premix[3]	5.0	5.0	5.0	5.0
Vitamin premix[4]	5.0	5.0	5.0	5.0
Salt	5.0	5.0	5.0	5.0
Titanium oxide	3.0	3.0	3.0	3.0
Analyzed chemical composition (DM basis)				
DM, %	89.3	81.8	88.3	91.4
CP, %	14.8	14.7	16.3	15.7
Phytic acid, g/kg	0.2	1.2	4.8	4.0
Ca, g/kg	8.37	8.95	8.43	11.04
Mg, g/kg	0.42	0.38	0.76	1.10
P, g/kg	4.48	5.51	7.59	7.63
Cu, mg/kg	69	77	130	136
Fe, mg/kg	241	279	437	415
Mn, mg/kg	41	62	92	94
Zn, mg/kg	124	155	157	157

[1]Nonstarch polysaccharide (NSP) fractions were supplemented as 5% actual NSP. CEL, cellulose; CMC, carboxymethylcellulose; LG, low viscous oat β-glucan; HG, high viscous oat β-glucan.

[2]Contained per kilogram of diet: Fe, 113 mg; and Mn, 5 mg.

[3]Provided per kilogram of diet: Zn, 100 mg as $ZnSO_4$; Fe, 80 mg as $FeSO_4$; Cu, 50 mg as $CuSO_4$; Mn, 25 mg as $MnSO_4$; I, 0.5 mg as $Ca(IO_3)_2$; and Se, 0.1 mg as Na_2SeO_3.

[4]Provided per kilogram of diet: vitamin A, 8,250 IU; vitamin D_3, 825 IU; vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; riboflavin, 5 mg; menadione, 4 mg; folic acid, 2 mg; thiamine, 1 mg; D-biotin, 0.2 mg; and vitamin B_{12}, 0.025 mg.

for each pig observation, stored at −20°C, subsampled, and freeze-dried before analyses. For analysis of SCFA and quantification of bacterial groups, freshly voided feces were stored at −20°C.

Analytical Methods

Diets and freeze-dried digesta and feces were finely ground in a centrifugal mill through a 1.0-mm mesh screen (Retsch, Haan, Germany) before analyses of DM (method 930.15; AOAC, 2006) and TiO_2 using spectrophotometry (method 975.21; AOAC, 2006). Phosphorus in feed, digesta, feces, and urine was analyzed spectrophotometrically at 400 nm using the vanadate-molybdate method (method 946.06; AOAC, 2006). The Ca, Mg, Cu, Fe, Mn, and Zn content in feed, digesta, feces, and urine was analyzed by dry ashing the samples (method 968.08; AOAC, 2006) followed by atomic absorption spectrometry (Varian SpectAA 240 FS, Mississauga, Ontario, Canada). The Ca and Mg content in feed, digesta, feces, and urine were analyzed after adequate dilution into 0.1% (wt/vol) lanthanum chloride. Phytic acid was determined at the University of Missouri, Columbia (method 986.11; AOAC, 2006). The 16S rRNA gene copy number of bacterial groups (i.e., total bacteria; *Lactobacillus* spp.; *Enterococcus* spp.; *Streptococcus* spp.; *Bifidobacterium* spp.; *Clostridium* cluster I, IV, and XIVa; *Bacteroides-Prevotella-Porphyromonas* group; and *Enterobacteriaceae*) in feces was quantified after phenol-chloroform DNA extraction (7500 Fast Real-Time PCR System, Applied Biosystems, Foster City, CA; Metzler-Zebeli et al., 2010). Feces were analyzed for SCFA (acetic, propionic, butyric, iso-butyric, valeric, iso-valeric, and caproic acid) by gas chromatography as described by Htoo et al. (2007).

Calculations and Statistical Analysis

Daily ileal flow and fecal excretion of minerals were defined as the amounts of minerals present in ileal digesta and feces. Amounts of minerals in digesta were calculated according to Eq. [1]:

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

where D_O is the daily total output of a mineral in ileal digesta or feces (g/d), A_I is the concentration of a mineral in ileal digesta or feces (g/d), I_D is the marker

concentration in the assay diet (g/d), I_I is the marker concentration in ileal digesta or feces (g/d), and F_I is the daily DMI (kg/d).

Daily postileal net mineral flux was defined as the net absorption or secretion of minerals in the large intestine as calculated from the difference between the ileal flow and the fecal excretion of minerals according to Eq. [2]:

$$D_{LI} = D_{Ileal\ digesta} - D_{Feces}, \quad [2]$$

where D_{LI} is the daily mineral flux in the large intestine (g/d), D_{Feces} is the mineral amount present in feces (g/d), and $D_{Ileal\ digesta}$ is the mineral amount present in ileal digesta (g/d).

Daily apparent retention was calculated as difference between daily mineral intake and mineral excretion in feces and urine according to Eq. [3]:

$$D_R = F_I - (E_F + E_U), \quad [3]$$

where D_R is the daily mineral retention (g/d), E_F is the mineral excretion in feces (g/d), and E_U is the mineral excretion in urine (g/d).

Data were analyzed by ANOVA using PROC MIXED (SAS Inst. Inc., Cary, NC). Because of digesta contamination in urine, 3 urine samples were excluded from analysis so that there were 6 observations for the CMC diet and 7 for the CEL diet. For the determination of any influential observation on the model, the Cook's distance test was used as the measure. Any observation having a Cook's distance greater than 0.5 was considered influential and hence deleted from further analysis. Fixed effects included pig and treatment effect. Period and pig within a square were considered random effects, assuming a compound symmetry variance-covariance structure (type = cs). Degrees of freedom were approximated using the Kenward-Rogers method (ddfm = kr). If the treatment effect was significant, means were separated using the probability of difference. A probability level of $P < 0.05$ was defined as significant. The relationships among variables of postileal mineral flux, fecal SCFA concentrations, and 16S rRNA gene copies of bacterial groups in feces were quantified using the weighted linear and nonlinear regression analysis (PROC REG, SAS). Predicted values of dependent variables after adjustment to the above-explained model (i.e., pig and period effects) were regressed to SCFA and number of bacterial groups in feces (independent variable) according to St-Pierre (2001).

RESULTS

Pigs recovered well from surgery and remained healthy throughout the study. The average BW of the pigs was 29.1, 37.8, 45.0, and 53.7 kg at the beginning of periods 1, 2, 3, and 4, respectively. The average BW of the pigs was 62.6 kg at the end of the study.

Diet Composition and Feed Intake

The LG and HG diets contained 58 and 50 g of β-glucans/kg of DM (data not shown). The CP content was 1 to 1.6% greater for the LG and HG diets compared with the CEL and CMC diets (Table 1). Although the amounts of Ca, Mg, P, Zn, Fe, Cu, and Mn originating from non-NSP components of the diet were similar for all 4 diets, the NSP fractions contributed differently to the mineral levels of the diets due to intrinsic minerals in CMC, LG, and HG fractions. As a result, the mineral content was greater for the CMC, LG, and HG diets compared with the CEL diet. Furthermore, the CMC, LG, and HG diets contained 1 to 3.8 g more phytic acid/kg of DM than the CEL diet. The average daily DMI across the 4 experimental periods was 1.30 ± 0.01 kg/d for pigs receiving the CEL and HG diets and 1.20 ± 0.01 kg/d for pigs fed the CMC and LG diets ($P < 0.001$; data not shown).

Mineral Balance

Because of the greater mineral content, pigs fed the LG and HG diets had greater ($P < 0.001$) intakes of Mg, P, Cu, Fe, Mn, and Zn compared with CEL and CMC, and pigs fed the HG diet had also a greater Ca intake ($P < 0.001$; Tables 2 and 3). Ileal flow and the apparent retention of macro- and trace minerals were affected by the NSP fractions ($P < 0.05$). In general, CMC reduced ($P < 0.05$) the ileal flow of Ca, Mg, P, Cu, Fe, and Mn and increased ($P < 0.05$) the apparent retention of Ca, Mg, and P compared with the other NSP fractions, whereas the CEL diet caused the least ($P < 0.05$) apparent retention of Ca, Mg, P, Cu, Fe, Mn, and Zn compared with CMC, LG, and HG.

Regression Analysis

Fecal SCFA were related to postileal flux of Ca, Mg, Cu, and Zn (Figures 1, 2, 3, and 4). Postileal Ca absorption was negatively related ($R^2 = 0.24$ to 0.45, $P < 0.05$) to fecal total SCFA and propionate concentrations when greater than 95.6 and 14.9 mmol/kg of DM, respectively. Postileal secretions of Cu and Zn decreased linearly ($R^2 = 0.94$ to 0.99, $P < 0.001$) with increasing total SCFA and acetate concentrations in feces. Among bacterial groups, only relations existed between the postileal Cu and Fe flux and the 16S rRNA copies for *Enterobacteriaceae* in feces (Figure 4). Postileal Fe secretion linearly increased ($R^2 = 0.20$, $P < 0.05$) with greater numbers of *Enterobacteriaceae* in feces, whereas Cu secretion was linearly reduced ($R^2 = 0.25$, $P < 0.01$) with greater 16S rRNA copy numbers of *Enterobacteriaceae*.

DISCUSSION

The results of the present study confirm our hypothesis that colonic fermentation influences the absorption

Table 2. Intake, ileal flow, fecal excretion, urinary excretion, and apparent retention of Ca, Mg, and P in pigs fed diets supplemented with cellulose (CEL), carboxymethylcellulose (CMC), low viscous oat β-glucan (LG), or high-viscous oat β-glucan (HG)[1]

	Low fermentable		High fermentable			
Item	Low viscous CEL	High viscous CMC	LG	HG	Pooled SEM	*P*-value
Ca						
Intake, g/d	10.92[b]	10.09[c]	10.25[c]	14.49[a]	0.06	<0.001
Ileal flow, g/d	14.93[ab]	5.39[c]	10.04[b]	16.79[a]	1.76	0.002
Fecal excretion, g/d	11.41[a]	3.58[b]	9.80[a]	12.34[a]	0.96	<0.001
Urinary excretion, g/d	0.39	0.29	0.31	0.24	0.09	0.686
Retention, g/d	−1.18[c]	5.60[a]	0.19[bc]	1.91[b]	0.10	0.005
Retention, % of intake	−7.0[b]	58.7[a]	3.2[b]	12.7[b]	9.9	0.009
Mg						
Intake, g/d	0.54[c]	0.45[c]	0.93[b]	1.44[a]	0.02	<0.001
Ileal flow, g/d	0.81[b]	0.21[d]	0.52[c]	1.49[a]	0.07	<0.001
Fecal excretion, g/d	0.48[b]	0.18[b]	0.50[b]	1.81[a]	0.11	<0.001
Urinary excretion, g/d	0.07[ab]	0.05[b]	0.06[b]	0.11[a]	0.02	0.047
Retention, g/d	0.01[b]	0.25[a]	0.35[a]	−0.38[c]	0.07	<0.001
Retention, % of intake	4.9[b]	50.6[a]	36.6[a]	−35.0[c]	8.0	<0.001
P						
Intake, g/d	5.85[d]	6.64[c]	9.30[b]	10.02[a]	0.09	<0.001
Ileal flow, g/d	5.07[b]	2.05[d]	3.29[c]	6.34[a]	0.41	<0.001
Fecal excretion, g/d	5.73[b]	1.57[d]	4.00[c]	7.32[a]	0.54	<0.001
Urinary excretion, g/d	0.12[c]	0.47[a]	0.27[bc]	0.36[ab]	0.08	0.068
Retention, g/d	0.04[c]	3.87[a]	5.17[a]	2.26[b]	0.62	0.001
Retention, % of intake	2.1[c]	50.4[a]	54.0[a]	23.0[b]	8.0	0.002

[a–d]Means in the same row with superscripts without a common letter differ, $P < 0.05$.

[1]Values are least squares means; n = 7, 6, 7, and 8 replicates for CEL, CMC, LG, and HG, respectively.

of minerals in the large intestine. Additionally, the 4 NSP fractions clearly and differently affected ileal flow and apparent retention of Ca, Mg, P, Cu, Fe, Mn, and Zn. For example, effects of viscosity on ileal flow and apparent retention were not consistent for the 2 high viscous NSP fractions, CMC and HG. In contrast, the effects of low viscous CEL on ileal flow were very similar to those of HG. However, because the mineral content among the diets differed, the obtained results must be interpreted with care and data should be only directly compared in cases with similar mineral intake. Intestinal absorption decreases, whereas fecal and urinary excretion of minerals increases with increasing dietary intake (Weigand and Kirchgessner, 1980; Rodehutscord et al., 1999). Therefore, data with respect to ileal flow, fecal and urinary excretion, and apparent retention of minerals in the present study does not exclude that homeostatic regulations occurred at greater mineral intake for LG and particularly for HG compared with CEL and CMC. However, the non-NSP components were consistent among the 4 diets so that the dissimilar mineral amounts were solely caused by the NSP supplements. In this respect, CMC, LG, and HG contained phytic acid so that probably a substantial part of the intrinsic minerals in these NSP fractions were chelated by phytic acid and therefore less available than the macro- and trace mineral supplements in the basal diet (NRC, 1998). For that reason, the approach selected for the present study to avoid compensation for differences in dietary mineral content seemed better than balancing the diets with inorganic mineral sources because of the greater digestibility of inorganic mineral sources compared with plant-derived minerals (NRC, 1998). Other mineral sources (e.g., dicalcium phosphate) also contributed to the dietary trace mineral concentrations. Trace minerals originating from feed ingredients other than the mineral premix were likely of less bioavailability compared with trace minerals in the mineral premix because the latter were in the form of sulfates with 100% bioavailability (NRC, 1998). For instance, Fe in dicalcium phosphate occurs in the form of Fe_2O_3 that is not available for the pig (NRC, 1998).

Daily Ca intake was marginally deficient for pigs fed the CMC, LG, and CEL diets, whereas pigs fed HG had a daily Ca intake that was approximately 25% above the daily requirement (NRC, 1998). The daily intake of Mg was also less than the daily requirement for pigs fed CEL and CMC, whereas intake was greater than the Mg requirement for pigs fed LG and HG (NRC, 1998). Considering the marginal deficient daily intake of Ca and Mg with the CEL and CMC diet, some Ca and Mg might be absorbed in the small intestine as the main site of macromineral absorption in pigs (Patience and Zijlstra, 2001; Schröder and Breves, 2006) because mineral absorption is negatively correlated with mineral intake (Guéguen and Pointillart, 2000; Coudray et al., 2005). However, more Ca and Mg were found in ileal digesta than were taken up from the diet in pigs

Table 3. Intake, ileal flow, fecal excretion, urinary excretion, and apparent retention of Cu, Fe, Mn, and Zn in pigs fed diets supplemented with cellulose (CEL), carboxymethylcellulose (CMC), low viscous oat β-glucan (LG), or high-viscous oat β-glucan (HG)[1]

	Low fermentable		High fermentable			
Item	Low viscous CEL	High viscous CMC	LG	HG	Pooled SEM	*P*-value
Cu						
Intake, mg/d	93[c]	93[c]	161[b]	176[a]	2	<0.001
Ileal flow, mg/d	81[a]	36[b]	48[b]	60[ab]	10	0.032
Fecal excretion, mg/d	154[a]	42[b]	141[a]	142[a]	13	<0.001
Urinary excretion, mg/d	1[b]	3[a]	2[a]	2[ab]	0.4	0.069
Retention, mg/d	−63[b]	49[a]	18[a]	35[a]	17	<0.001
Retention, % of intake	−63.5[b]	58.0[a]	13.8[bc]	20.6[c]	5.5	0.069
Fe						
Intake, mg/d	317[b]	336[b]	533[a]	545[a]	6	<0.001
Ileal flow, mg/d	617[a]	261[b]	497[a]	681[a]	66	0.003
Fecal excretion, mg/d	857[a]	442[b]	615[ab]	706[ab]	90	0.037
Urinary excretion, mg/d	2[b]	28[a]	7[b]	3[b]	6	0.052
Retention, mg/d	−500[b]	−138[ab]	−65[a]	−164[ab]	132	0.097
Retention, % of intake	−149.0[b]	−42.9[a]	−6.0[a]	−26.6[a]	30	0.006
Mn						
Intake, mg/d	53[d]	75[c]	113[b]	124[a]	2	<0.001
Ileal flow, mg/d	116[a]	32[b]	65[b]	77[ab]	15	0.012
Fecal excretion, mg/d	133[ab]	44[c]	117[b]	159[a]	11	<0.001
Urinary excretion, mg/d	1[b]	5[a]	2[b]	1[b]	0.5	0.008
Retention, mg/d	−77[c]	23[a]	−5[ab]	−35[b]	6	0.119
Retention, % of intake	−140.3[b]	19.2[a]	−5.6[a]	−26.8[a]	15.2	<0.001
Zn						
Intake, mg/d	162[d]	187[c]	192[b]	206[a]	1	<0.001
Ileal flow, mg/d	179[ab]	111[b]	143[ab]	256[a]	32	0.018
Fecal excretion, mg/d	321[a]	151[b]	251[a]	293[a]	24	0.001
Urinary excretion, mg/d	12[c]	62[a]	27[b]	12[c]	8	0.005
Retention, mg/d	−166[c]	−33[a]	−87[b]	−98[b]	15	0.220
Retention, % of intake	−102.2[b]	−37.2[a]	−46.2[a]	−45.0[a]	20.4	0.112

[a–d]Means in the same row without a common superscript differ ($P < 0.05$).

[1]Values are least squares means, n = 7, 6, 7, and 8 replicates for CEL, CMC, LG, and HG, respectively.

fed the CEL diet, whereas 47% of the Ca ingested in pigs fed the CMC diet was digested at the ileal level. Therefore, the daily requirement of Ca and Mg seemed to be greater in pigs fed diets supplemented with CEL because of negative effects of CEL on absorption in the small intestine compared with CMC. Likewise, pigs receiving the HG diet seemed to have a greater requirement for Ca and Mg; the ileal flow of Ca and Mg surpassed the intake too. Similarly, the ileal flow of Fe and Zn was surpassing the daily intake for CEL and HG and the ileal flow of Mn was also greater than the daily intake for HG. When comparing daily intake and ileal flow of P, 2.9 g/d more P was absorbed in the small intestine of pigs fed the HG diet that contained the most P than in pigs fed the CEL diet that contained the least P, indicating that the dietary P content was not the only factor influencing small intestinal P absorption. Intestinal P absorption is negatively related to dietary P content (Rodehutscord et al., 1999) and is greatest with a dietary P content below the actual P requirement. Hence, aside from the dietary mineral content, the NSP fractions obviously drastically affected the mineral absorption in the upper GIT. Effects on ileal mineral flow may be related to the impact of the NSP fractions on digesta passage rate, ion-binding capacity, and increased digesta viscosity (Idouraine et al., 1996; Lentle and Jansson, 2008). In this respect, CEL shortened the retention time in the small intestine, thereby reducing time for nutrient digestion and absorption, whereas CMC increased retention time because of an increased digesta viscosity (Hooda et al., 2010b). Additionally, the NSP fractions may have increased the endogenous mineral losses in the upper GIT by increasing gastroduodenal, pancreatic, and bile secretions (Dierick et al., 1989; Malecki et al., 1996; Krebs, 2000).

Different amounts of minerals reached the large intestine because of the varying ileal flow among the 4 diets. Beside minerals, more fermentable substrate entered the large intestine with CEL (308 g of DM/kg of DMI), LG (242 g of DM/kg of DMI), and HG (277 g of DM/kg of DMI) compared with CMC (165 g of DM/kg of DMI), thereby stimulating fermentation in the hindgut of these pigs (Metzler-Zebeli et al., 2010). This finding is in line with observations obtained in rats where highly fermentable carbohydrates, such as pectin, lactulose, and resistant starch, increased the ce-

cal pool of minerals by stimulating fermentation in the hindgut (Demigné et al., 1989). Although the role of the large intestine in Ca absorption is assumed to be small in pigs (Schröder and Breves, 2006), the present data indicate that the large intestine may become the main site for Ca absorption when NSP fractions are included in the diet that interfere with cation absorption in the small intestine. In fact, the postileal net Ca absorption of 3.5 and 4.5 g/d for CEL and HG and a net Mg absorption of 0.33 g/d for CEL greatly support that the large intestine may compensate for the reduced cation absorption in the small intestine (Abrams et al., 2007; Patterson et al., 2008). Although the underlying mechanisms are not completely understood, the present data confirm results obtained in studies with rats and humans receiving diets containing fructans. Fer-

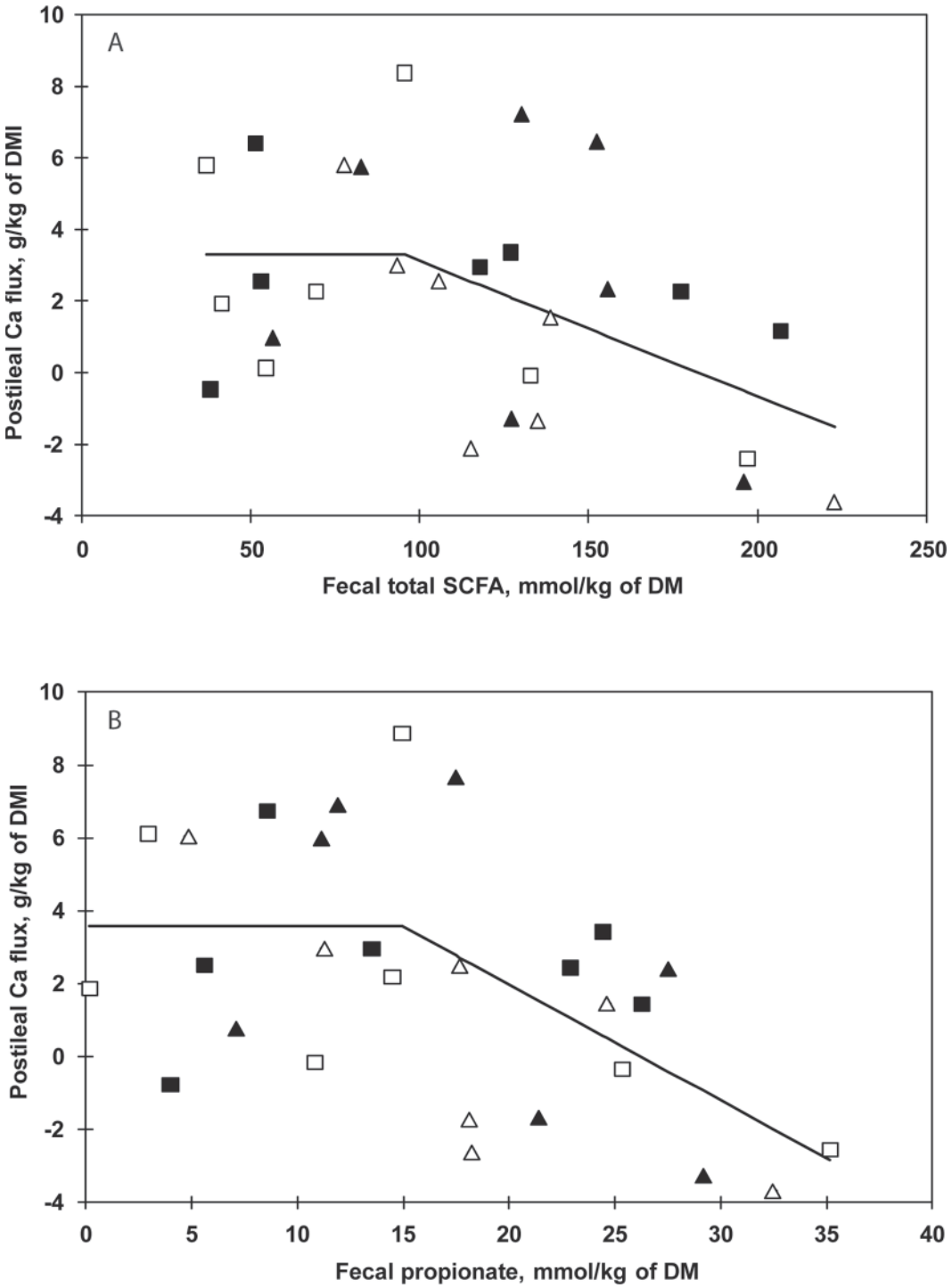

Figure 1. Relation between postileal net Ca flux (y) and concentration (x) of total short-chain fatty acids (SCFA; A) and propionate (B) in feces of pigs fed diets supplemented with cellulose (■), carboxymethylcellulose (□), low viscous oat β-glucan (△), or high viscous oat β-glucan (▲). A: $y = 6.915 - 0.0379 \times x$, if $x \leq \pi$ [$\pi = 95.6$ mmol/kg of DM, asymptotic plateau of $y = 3.293$ g/kg of DMI, root mean square error (RMSE) $= 2.939$, $R^2 = 0.24$, and $P = 0.031$]; B: $y = -0.162 + 0.030 \times x$, if $x \leq \pi$ ($\pi = 14.9$ mmol/kg of DM, asymptotic plateau of $y = 3.574$ g/kg of DMI, RMSE $= 2.695$, $R^2 = 0.45$, and $P < 0.001$). Values are least squares means; n = 7 per treatment.

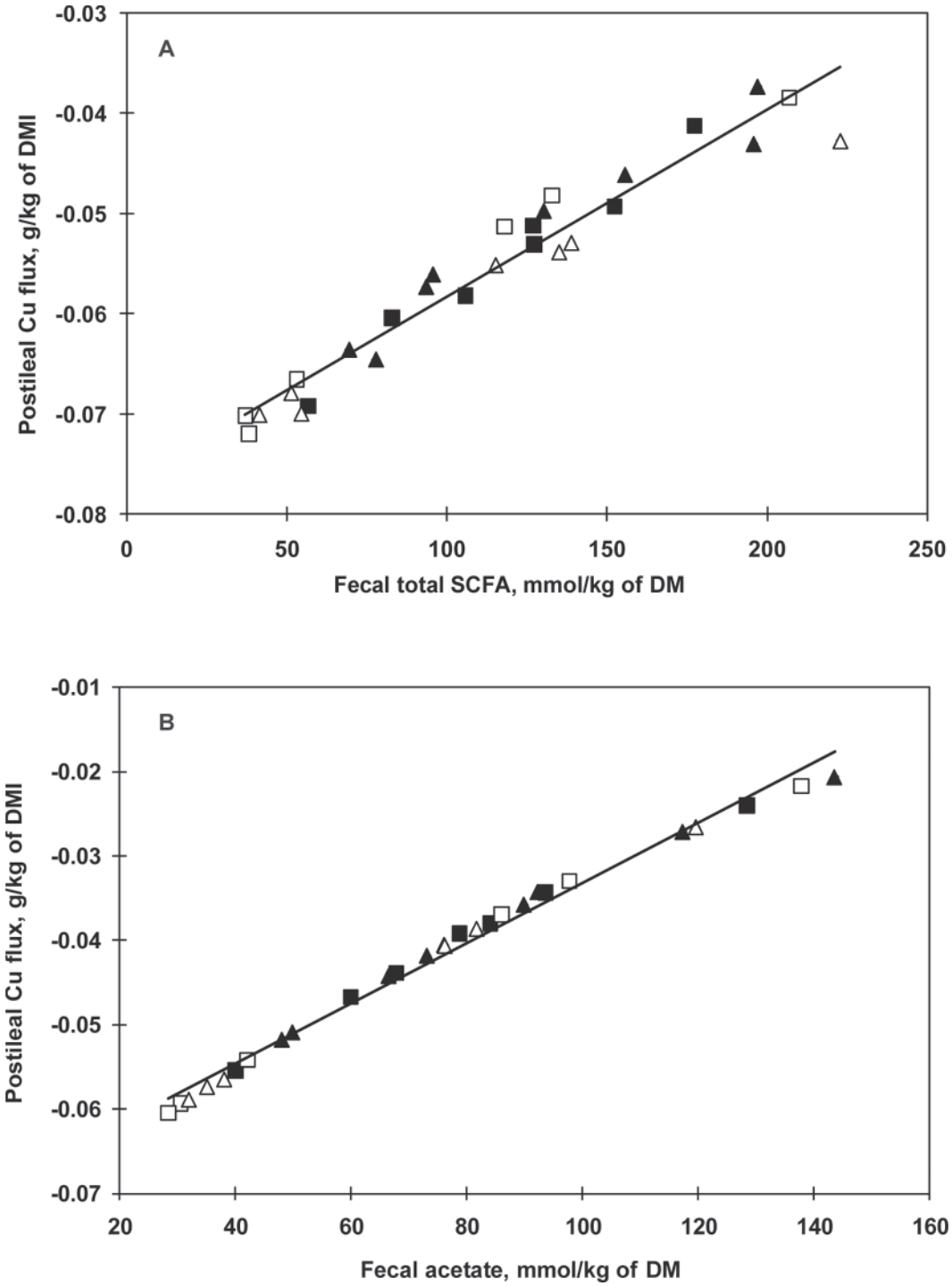

Figure 2. Relation between postileal net Cu flux (y) and concentration (x) of total short-chain fatty acids (SCFA; A) and acetate (B) in feces of pigs fed diets supplemented with cellulose (CEL; ■), carboxymethylcellulose (CMC; □), low viscous oat β-glucan (LG; △), or high viscous oat β-glucan (HG; ▲). A: $y = -0.077 + 0.0002 \times x$, root mean square error (RMSE) = 0.003, $R^2 = 0.94$, $P < 0.001$; B: $y = -0.069 + 0.0004 \times x$, RMSE = 0.001, $R^2 = 0.99$, and $P < 0.001$. Values are least squares means; n = 7, 6, 7, and 8 for CEL, CMC, LG, and HG, respectively.

mentation in the large intestine may enhance postileal cation absorption by increasing the solubility of cations by acidifying the colonic content or by a direct effect of SCFA (Abrams et al., 2007; Demigné et al., 2008). The fact that colonic fermentation may have affected the postileal net mineral flux was strongly supported by the relationships between postileal net mineral flux and fecal SCFA in pigs that were found using regression analysis. However, increasing total SCFA in feces were negatively related to postileal net Ca absorption, indicating that Ca may also have been needed to buffer the intestinal lumen with progressing fermentation. Calcium salts, especially Ca-phosphate, play an important role in the control of colonic pH to neutralize excessive acidification because of microbial metabolism (Demigné et al., 1989). Therefore, increased colonic fermentation may have induced sufficient Ca to reach the colon to buffer intestinal pH. For instance, the amount

of Ca excreted in feces was similar for CEL, LG, and HG with 10 to 12 g/d. Given that the amount of DM fermented in the large intestine (Metzler-Zebeli et al., 2010) was also comparable among CEL (129 g of DM/kg of DMI), LG (102 g of DM/kg of DMI), and HG (126 g of DM/kg of DMI) and drastically greater than CMC (23 g of DM/kg of DMI) that induced less DM fermentation, the colonic content of Ca and the need to buffer the luminal pH might be linked.

In general, postileal secretion of trace minerals adjusts the body mineral pool when initially too large amounts were absorbed in the small intestine (King et al., 2000). This may partly explain the postileal net secretion of Cu, Fe, Mn, and Zn observed in the present

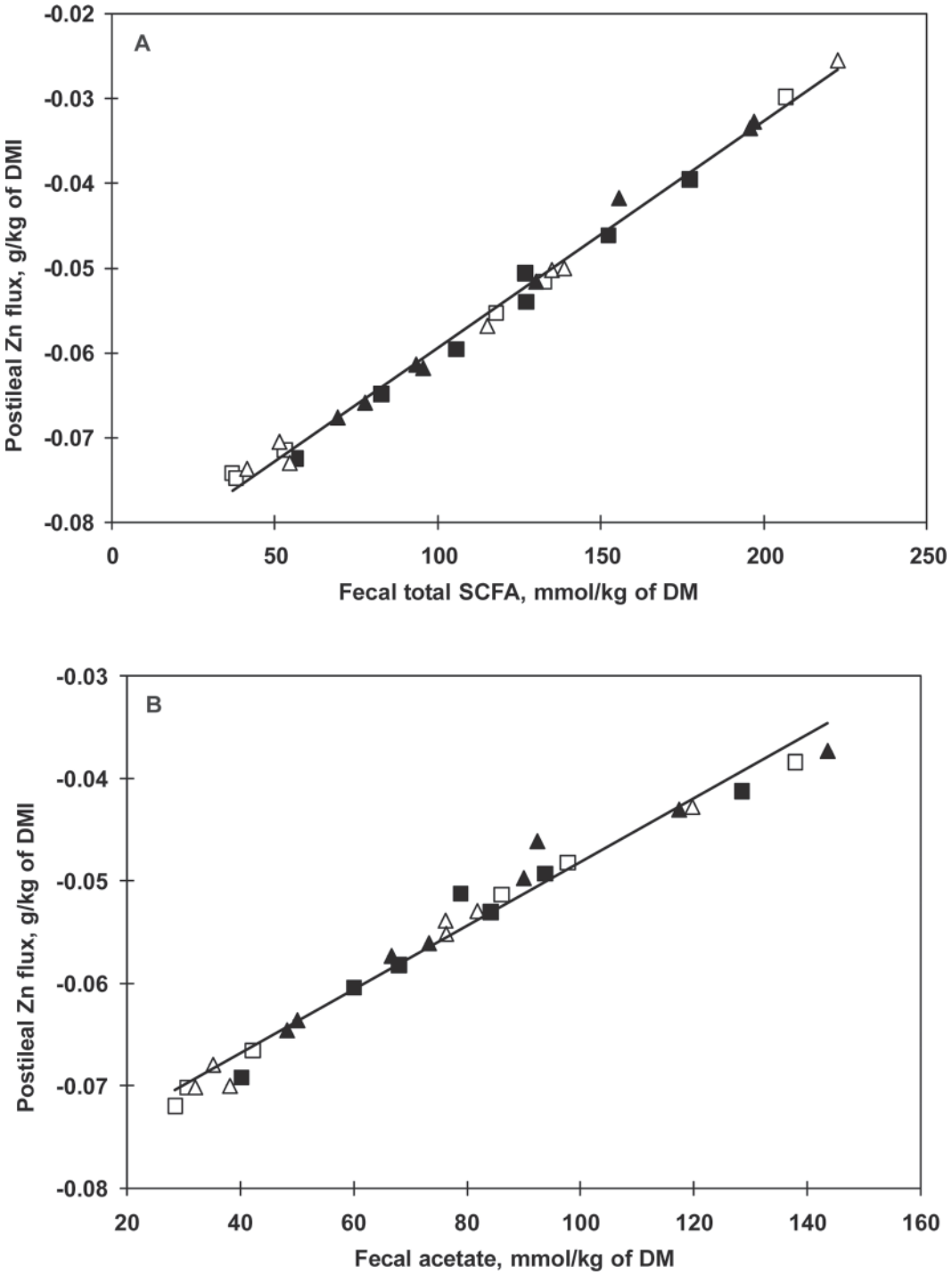

Figure 3. Relation between postileal net Zn flux (y) and concentration (x) of total short-chain fatty acids (SCFA; A) and acetate (B) in feces of pigs fed diets supplemented with cellulose (CEL; ■), carboxymethylcellulose (CMC, □), low viscous oat β-glucan (LG; △), or high viscous oat β-glucan (HG; ▲). The best fit, a linear regression; A: $y = -0.0862 + 0.0003 \times x$, root mean square error (RMSE) = 0.001, $R^2 = 0.99$, $P < 0.001$; B: $y = -0.079 + 0.0003 \times x$, RMSE = 0.002, $R^2 = 0.97$, $P < 0.001$. Values are least squares means; n = 7, 6, 7, 8 for CEL, CMC, LG, and HG, respectively.

study, but other factors, such as bacterial requirements for growth and fermentation (Hrdina et al., 2009) likely played a role as well, particularly for those trace minerals where the ileal flow surpassed the daily intake. When regressing the trace mineral secretion with fecal SCFA, the postileal net Zn and Cu secretion decreased with increasing concentrations of SCFA in feces. Besides the coupling of colonic uptake of SCFA with cations via, most probably, a cation-proton antiporter, the uptake of cations may be also related to specific SCFA (Mineo et al., 2001, 2006). In the present study, fecal acetate was related to net Zn and Cu secretion. The SCFA are an important factor in the stimulation of epithelial cells and promotion of cell proliferation in the large intestine (Mineo et al., 2006), thereby inducing colonic enlargement (Serena et al., 2008). Other mechanisms for SCFA to affect active cation absorption might be via influencing gene expression of proteins involved in mucosal mineral binding and sequestration (Tako et al., 2008) or modifying colonic mucosal permeability (Mineo et al., 2006).

Like the host animal, hindgut bacteria also have special mineral requirements (Durand and Komisarczuk, 1988; Groot et al., 2005). Bacteria may therefore

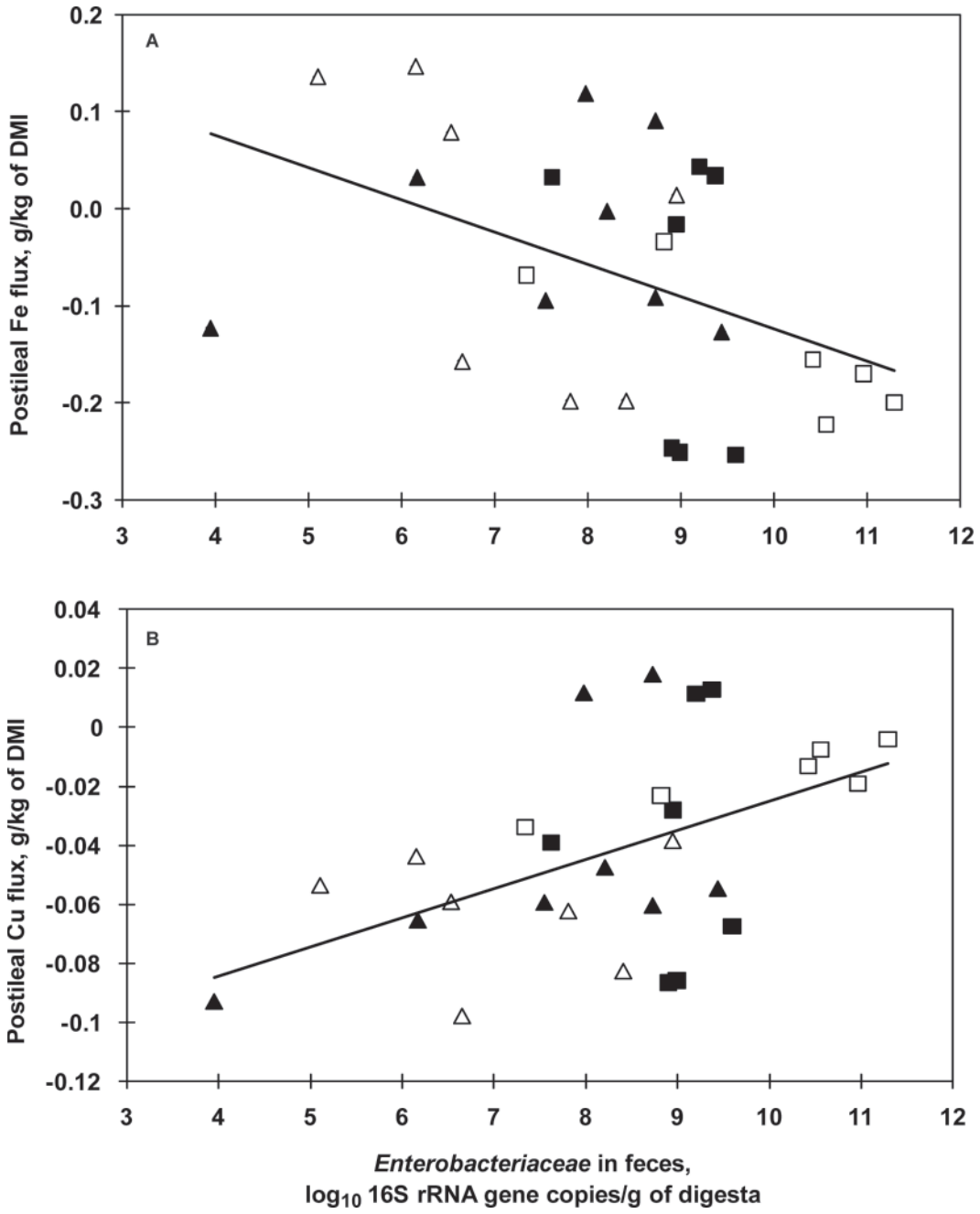

Figure 4. Relation between postileal net Fe (A) and Cu (B) flux (y; g/kg of DMI) and the fecal number of *Enterobacteriaceae* (x; $\log_{10}$ 16S rRNA gene copies/g of digesta) in pigs fed diets supplemented with cellulose (CEL; ■), carboxymethylcellulose (CMC; □), low viscous oat β-glucan (LG; △), or high viscous oat β-glucan (HG; ▲). The best fit, a linear regression; A: Fe = 0.208 − 0.0332 × x, root mean square error (RMSE) = 0.116, R^2 = 0.20, P = 0.017, and n = 28; B: Cu = −0.124 + 0.010 × x, RMSE = 0.030, R^2 = 0.25, and P = 0.007. Values are least squares means; n = 7, 6, 7, 8 for CEL, CMC, LG, and HG, respectively.

compete with the host to fulfill their own mineral requirements (Hrdina et al., 2009; Metzler et al., 2009). Minerals are lost for the host after incorporation into bacteria and will be excreted in feces. Thus, the postileal net secretion of Mg for HG and Cu, Fe, Mn, and Zn for all diets may be not only linked to an excessive dietary intake but also related to bacterial growth. To reveal whether the postileal net mineral flux could be directly linked to bacterial groups in the present study, the postileal net mineral flux was regressed with bacterial gene copy numbers in feces. Both the postileal net flux of Fe and Cu was correlated with 16S rRNA gene copies of *Enterobacteriaceae* in feces. The large number of 16S rRNA gene copies for *Enterobacteriaceae* in pigs fed the CMC diet is similar with viscous CMC stimulating growth of *Escherichia coli* in piglets (McDonald et al., 2001). Iron is the first growth-limiting nutrient for *E. coli* (Appenzeller et al., 2005), the dominant bacterium within the *Enterobacteriaceae* family in pigs (Leser et al., 2002). These bacteria likely benefited from the availability of unabsorbed dietary Fe. Because *Enterobacteriaceae* stimulate the immune response of the host (Weinberg, 1978), greater numbers of *Enterobacteriaceae* may have altered the postileal net Fe flux in such a way that even more Fe became available for bacterial growth in the intestinal lumen. The body withdraws the systemic Fe available for bacterial growth as part of the nonspecific defense mechanism against infection (Weinberg, 1978) so that less Fe is absorbed and more is secreted endogenously. Similarly, plasma Cu increases during an infection because Cu is necessary for an effective immune response (Ilbäck and Friman, 2007). Thus, the reduced intestinal net secretion of Cu might have been also linked to an immune response to increasing numbers of *Enterobacteriaceae*.

Because of greater ileal flow and postileal net secretion of minerals, CEL caused the greatest fecal excretion and least retention for all minerals that was negative except for Mg and P, although pigs fed the CEL diet had a markedly reduced mineral intake than pigs fed the LG and HG diets. This finding strongly indicates that the dietary inclusion of CEL increased the daily mineral requirement of growing pigs. However, the microcrystalline CEL used may act differently than cellulose naturally found in the fiber matrix of feedstuffs. Because the pigs were fed the diets for 10 d before starting feces and ileal digesta collection, the negative retentions for Fe, Mn, and Zn in pigs fed the CMC, LG, and HG diets might be partly related to homeostatic regulation due to the prolonged uptake of very large trace mineral content. Interestingly, the apparent retention of Cu was positive, ranging from 14 to 58% for CMC, LG, and HG, although the daily intake exceeded the actual daily requirement by 10 to 22 times (NRC, 1998).

In conclusion, the present data indicate that effects of the NSP fractions on mineral absorption were not limited to the small intestine but were also related to colonic fermentation, thereby confirming observations made in humans and rats that bacterial fermentation in the large intestine can influence colonic mineral absorption. Ileal mineral flow was increased and apparent retention was depressed when CEL was included in the diet, indicating that the actual requirement of minerals increased with this NSP. The large intestine may partly compensate for reduced mineral absorption in the small intestine. Additionally, fermentation of NSP and other dietary constituents flowing into the large intestine may stimulate net absorption or secretion of minerals depending on the mineral present and the fermentation intensity.

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