Virginiamycin improves phosphorus digestibility and utilization by growing-finishing pigs fed a phosphorus-deficient, corn-soybean meal diet[1,2]

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ABSTRACT: Evaluations of the nutritional effect of antibiotics have largely centered on effects related to the digestibility and utilization of protein and energy. The current study evaluated the potential effect of virginiamycin (VIR) on P digestibility in swine. A total of 70 barrows (mean initial BW = 51 to 64 kg) were used in 4 nutrient-balance experiments. A basal, corn-soybean meal diet that was not supplemented with any inorganic source of P was used in each experiment. In Exp. 1, two diets were tested: basal vs. basal plus 11 mg/kg of VIR. In Exp. 2, four diets were used with a 2 × 2 factorial arrangement of 0 and 11 mg/kg of VIR and 0 and 750 phytase (PHY) units/kg of diet (PU/kg). Experiments 3 and 4 were the same as Exp. 2, except PHY was reduced to 300 PU/kg. For all experiments, VIR improved P digestibility (32.71 to 37.72%, $P < 0.001$) and Ca digestibility (54.99 to 58.30%, $P = 0.002$). The addition of PHY improved both P and Ca digestibility ($P < 0.001$); 750 PU/kg increased P digestibility 27.3% (from 34.6 to 61.9%, $P < 0.001$), whereas 300 PU increased it 13.8% (from 33.4 to 47.2%, $P < 0.001$). In an experiment conducted to evaluate the long-term effects of VIR on gut microbial profile, pigs (24 gilts and 8 barrows; mean BW = 29.1 ± 0.50 kg) were fed a simple corn-soybean meal diet for 16 wk with a 2 × 2 factorial arrangement of VIR (0 and 11 mg/kg) addition and 0.15% dicalcium phosphate deletion. The long-term feeding of VIR in both the control diet and the diet with a marginally reduced P level resulted in a change in ileal microbial profile. A positive numerical increment in the number of phytate-utilizing bacteria was observed in both the normal and P-deleted diets (log unit increments of 12.4 and 17.2% over the respective controls, $P = 0.13$) when VIR was added. The addition of VIR also tended to affect lactobacilli populations (main effect, $P = 0.11$; interaction, $P = 0.02$); VIR decreased lactobacilli in the normal-P diet but did not affect this bacterial population in the P-deleted diet. In conclusion, the antibiotic VIR improves both Ca and P digestibility in pigs. The increase in digestibility is not as great as that provided by PHY, but because the potential mechanism of action (altered microbial populations) differs from that of PHY (direct addition of an enzyme), there can be a degree of additivity in P digestibility improvement when both products are used.

Key words: digestibility, phosphorus, phytase, pig, virginiamycin

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INTRODUCTION

Farm animals have been fed subtherapeutic levels of antibiotics as growth promoters for half a century. Although the mode of action of antibiotics on growth is not totally understood, their positive effect on energy and N utilization has been observed in several animal species, including swine (Vervaeke et al., 1979; Dierick et al., 1986). Evaluations of the nutritional effect of subtherapeutic antibiotic supplementation have centered on effects related to the digestibility and utilization of protein and energy, because these items constitute the greatest physical and economic portion of diets. Some studies have addressed the effects of antibiotics on mineral utilization in poultry (Lindblad et al., 1954; Brown, 1957; Buresh et al., 1985), but these evaluations are limited in swine. Reports with broilers indicate that virginiamycin (**VIR**) has potential to improve P digest-

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ibility. Buresh et al. (1985) reported that 22 mg/kg of VIR improved P utilization in chicks fed a corn-soybean-meal (**SBM**) diet containing 0.47% total P. At this P level, the VIR-added diet resulted in a 4.6% increase in tibia ash compared with the same diet without VIR. Schutte et al. (1994) reported that 20 mg/kg of VIR improved ileal P digestibility by 5.5 percentage units in ileostomized roosters fed a practical diet.

Because P is the most expensive mineral in swine diets, and it is associated with environmental issues, it seemed prudent to evaluate the potential effect of VIR on P digestibility in swine. The primary objective of these experiments was to evaluate VIR and phytase (**PHY**) effects on digestibility and on the retention and excretion of nutrients (particularly P) in growing pigs fed a P-deficient diet. This was accomplished by conducting 4 balance experiments utilizing the total collection method. An additional experiment to evaluate the long-term effects of VIR on gut microbiological profile was also conducted.

MATERIALS AND METHODS

Animals and Housing Conditions for Digestibility Experiments

All experiments were conducted under protocols approved by the University of Kentucky Institutional Animal Care and Use Committee.

A total of 70 growing-finishing barrows [Hampshire or Duroc × (Yorkshire × Landrace)] were used in 4 experiments. In each experiment, sibling pigs of similar BW within a replicate were allocated to treatments; half-siblings (i.e., a common sire) were used when enough full-siblings were not available.

In Exp. 1, sixteen barrows were fed a common grower diet for a minimum of 2 wk followed by a low-P corn-SBM-basal diet for a minimum of 10 d to standardize gastrointestinal tract conditions among the animals. Ten pigs were then selected based on overall condition, paired by genetic background, and randomly allotted within pair to 1 of 2 dietary treatments and placed in stainless steel metabolism crates. Animals had a 7-d adaptation (mean initial BW = 57.3 kg) to the crate and diet, followed by a 5-d total feces and urine collection phase. After the collection phase, the pigs were switched to the alternate dietary treatment, moved to group pens (1.22 × 2.44 m) with 2 to 3 pigs/pen for a 3-d respite from the metabolism crates. Pigs were then moved back into the crates for a repeat of the adaptation and collection procedures.

In Exp. 2, twenty-four barrows were allowed ad libitum access to the basal diet for 10 d before being allotted to 1 of 4 dietary treatments and beginning the 7-d adaptation period (mean initial BW = 63.7 kg), followed by the 5-d total collection phase.

In Exp. 3, twelve barrows were allowed ad libitum access to the basal diet for 9 d before being allotted to 1 of 4 dietary treatments and beginning the 7-d adaptation period (mean initial BW = 51.3 kg), followed by the 5-d total collection phase. As in Exp. 1, after the collection phase, the pigs were switched to the basal diet and moved to group pens (1.22 × 2.44 m) with 2 to 3 pigs/pen for a 4-d respite from the metabolism crates. Then, pigs were assigned to an alternate dietary treatment and moved back into the crates for a repeat of the adaptation and collection procedures.

In Exp. 4, twenty-four barrows were allowed ad libitum access to the basal diet for 4 d before being allotted to 1 of 4 dietary treatments and beginning the 7-d adaptation period (mean initial BW = 52.6 kg), followed by the 5-d total collection phase. For Exp. 2, 3, and 4, feeding management during the adaptation and collection phases was the same as in Exp. 1.

Table 1. Basal diet used in Exp. 1, 2, 3, and 4 (as-fed basis)

Item	%
Ingredient	
Corn, ground	74.80
Soybean meal, 48% CP	23.30
Vitamin mix[1]	0.075
Trace mineral mix[2]	0.075
Limestone, ground	1.40
Phosphate source	0.00
Salt	0.35
Total	100.00
Calculated composition	
CP, %	17.28
Lys, %	0.90
ME, kcal/kg	3,346
Ca, %	0.60
P, total, %	0.37
P, available, %	0.07

[1]The premix supplied the following ingredients per kilogram of diet: 4,950 IU of vitamin A, 660 IU of vitamin D_3, 33 IU of vitamin E, 4.8 mg of vitamin K (as menadione sodium bisulfite complex), 6.6 mg of riboflavin, 16.5 mg of pantothenic acid, 33.0 mg of niacin, 0.99 mg of folic acid, 0.165 mg of D-biotin, 24.5 μg of vitamin B_{12}, and 3.3 mg of vitamin B_6.

[2]The premix supplied the following ingredients per kilogram of diet: 120 mg of Fe (iron sulfate monohydrate), 150 mg of Zn (zinc oxide), 45 mg of Mn (manganous oxide), 12 mg of Cu (copper sulfate pentahydrate), 1.5 mg of I (calcium iodate), and 0.3 mg of Se (sodium selenite).

Dietary Treatments for Digestibility Experiments

A basal, corn-SBM diet, which was not supplemented with any inorganic source of P (Table 1), was prepared separately for each experiment. The basal diet met or exceeded all NRC (1998) nutrient requirement estimates, with the exception of P. In Exp. 1, two diets were tested: the basal diet vs. basal supplemented with VIR (Stafac 20, Phibro Animal Health Co., Fairfield, NJ). The VIR was included at 11 mg/kg, a level approved for growth promotion purposes in swine. In Exp. 2, a total of 4 diets were used with a 2 × 2 factorial arrangement of VIR and PHY (Natuphos 1200G, BASF Corp., Mount Olive, NJ). Inclusion rates of the 2 factors were 0 and 11 mg/kg of VIR and 0 and 750 PHY units (**PU**)/kg of diet. Exp. 3 also used a 2 × 2 factorial arrangement,

Table 2. Expected and analyzed phytase (PHY) and virginiamycin (VIR) levels in the diets (as-fed basis)

		PHY, unit/kg		VIR, mg/kg	
Exp.	Treatment	Expected	Analyzed[1]	Expected	Analyzed[2]
1	1	—	—	0	ND[3]
	2	—	—	11	10.7
2	1	0	ND	0	ND
	2	0	ND	11	13.7
	3	750	962	0	ND
	4	750	1,410	11	9.8
3	1	0	ND	0	ND
	2	0	ND	11	8.9
	3	300	420	0	ND
	4	300	286	11	7.4
4	1	0	ND	0	ND
	2	0	ND	11	9.4
	3	300	448	0	ND
	4	300	407	11	9.7
All	PHY premix, phytase units/g	1,200	1,326	—	—

[1]Average values of samples analyzed for both collections in each experiment. The limit of detection was 70 units/kg of diet; all unsupplemented diets were below that detection limit.
[2]The limit of detection was 2 mg/kg; all unsupplemented diets were below that detection limit.
[3]ND = not detected.

with the only difference from Exp. 2 being that the intended PHY level was reduced from 750 to 300 PU/kg. The VIR and PHY premixes used in Exp. 1 to 3 came from the same bag of commercial product, with the composition label for each product used to determine the dietary inclusion rate. Experiment 4 was a repetition of Exp. 3; the PHY premix was analyzed before Exp. 4, and the result was a PHY level of 1,326 PU/g, which was the value used to calculate PHY concentration in Exp. 4 instead of the minimum value of 1,200 PU/g on the label. Actual analyzed values for the dietary treatments are provided in Table 2.

For each experiment, large quantities of the basal diet were mixed, to which the additives (VIR or PHY) were applied; this prevented differences in nontreatment components of the diets. In Exp. 2 and 3, the diet that contained both amendments was made by weighing out the appropriate amount of each amendment. In Exp. 4, a single batch of the basal diet was prepared and then divided into 4 fractions. One of the quarter fractions was blended with VIR, and another one was blended with PHY, with the blending of both additives (VIR or PHY) done in a proportion equivalent to twice the desired concentration for the final experimental diets (i.e., 22 mg/kg of VIR and 600 PU of PHY/kg). To make experimental diets 2 and 3, a part of each concentrated portion was blended with an equal amount of the unblended basal diet. Diet 4 was prepared by blending together the same amounts of both concentrated fractions.

Adaptation and Collection Procedures for Digestibility Experiments

Once the pigs were placed in the metabolism crates, the diets were weighed as individual meals into labeled plastic bags and were separated by treatment. Pigs were fed at 3% of BW during the experiments, in a gruel form, with the feed divided into 2 daily meals. The beginning and end of the collection period were marked by the addition of 0.5% indigo carmine to the morning meal. After consumption of each meal, water was added to the metabolism crate feeder to allow ad libitum access between meals. During the collection periods, the total amounts of feces were collected daily, stored in plastic bags, and frozen at –20°C until the end of the collection period. The collection of urine was initiated 14 h after the feeding of the first marked meal and was completed 14 h after the feeding of the second marked meal at the end of the collection period. A total of 150 mL of 3 *N* HCl was added to the collection container at the beginning of each collection to prevent volatilization of urinary N. Urine was collected every 24 h and stored at –20°C.

Sample Preparation for Digestibility Experiments

To obtain a representative sample of urine for nutrient analysis, the collected samples were thawed at room temperature and proportionally composited by weight for each pig according to the recorded daily excretion. Composited samples remained frozen at all times until analysis.

All frozen feces were dried in a forced-air oven (Tru-Temp, Hotpack Corp., Philadelphia, PA) at 55°C for 1 wk, then air-equilibrated, weighed, and ground using a Wiley laboratory mill (model 3, Arthur H. Thomas Co., Philadelphia, PA) through a 1-mm screen. All ground feces from each collection period were thoroughly mixed in a single bag for each pig. From this bag, a sample for chemical analysis was obtained and reground using a smaller high-speed grinder (type

4041, model KSM 2-4, Braun Inc., Woburn, MA). After being composited, feces were kept in a cold room at 4 to 8°C until chemical analysis.

Laboratory Analysis for Digestibility Experiments

Feces and feed were analyzed for DM content. Feces, feed, and urine were analyzed for energy, N, P, and Ca concentration; concentrations of Mg, K, Mn, Zn, Fe, Cu, and Na were assessed in all experiments, except in Exp. 3. Total contents of nutrients in feces, urine, and feed were calculated as the product of nutrient concentration and the total amount of material. Samples were analyzed at least in duplicate, and analysis was repeated when abnormal variation was observed (generally when the CV was >5%).

Dry matter in feed and feces was assessed according to an adaptation of the AOAC (1995a) method involving overnight drying (105°C) of the samples in a convection oven (Precision Scientific Co., Chicago, IL) and then calculating moisture contents as the difference between weighings. Gross energy contents were assessed by bomb calorimetry, consisting of the ignition of samples in a pressurized-O_2 environment, and measuring the heat of combustion as the amount of energy transferred to a known mass of water contained in the calorimeter (model 1261 isoperibol bomb calorimeter, Parr Instruments Co., Moline, IL). Benzoic acid pellets with known combustion heat were ignited at the beginning and end of each set of samples to verify calorimeter calibration. Feed and feces samples were assessed in duplicate by a procedure adapted from AOAC (1995a). To measure urine energy, samples were oven-dried for 2 d at 55°C into polyethylene flat bags (Jeb Plastics Inc., Wilmington, DE) before combustion. To obtain the urinary energy contents, the known heat of combustion per gram of bag material was subtracted from the total heat measured.

Nitrogen was measured using Dumas methodology in an automatic N analyzer (model FP-2000, LECO Corp., Saint Joseph, MI). Ignition of blanks and EDTA samples with known N contents was conducted daily to calibrate the equipment and to check for drift in the readings.

Phosphorus in feed and feces was assessed by a gravimetric method (modification of method 968.08; AOAC, 1990), in which samples were weighed, ashed, acid-digested, diluted to 250 mL and then reacting 50 mL of the liquid with Quimociac solution, filtered, and the precipitate obtained was weighed to calculate the P concentration. Phosphorus concentration in urine was assessed as inorganic P by a colorimetric procedure (procedure number 360-UVP, Sigma Diagnostics, St. Louis, MO) using a spectrophotometer (model Ultrospec IIE, 4057 UV/visible, LKB Biochrom Ltd., Cambridge, UK). The concentration was measured under UV light at 340 nm. A commercial reagent was used (ammonium molybdate, 0.40 mmol/L in sulfuric acid with surfactant; catalog number 360-3, Sigma Diagnostics) along with a set of 3 standards containing 1, 5, and 15 mg of P/dL (Ca/P Standard, catalog number 360-5, Sigma Diagnostics).

Except for P, all other mineral elements were assessed by flame atomic absorption spectrophotometry (Thermoelemental, SOLAAR M5, Thermo Electron Corp., Verona, WI) according to a modification of the AOAC procedure (method 927.02, 1995b).

Experimental Design and Statistical Analysis for the Digestibility Experiments

Experiment 1 challenged the null hypothesis of no effect of VIR on mineral digestibility, using 10 replications. A crossover design was used, in which a single group of 10 pigs (5 pairs by ancestry) was used in 2 collections, with each pig receiving a different treatment in each collection. The treatment structure was a 1-way treatment classification, and the experimental unit was the pig. The ANOVA was done using the GLM procedure (SAS Inst. Inc., Cary, NC). The model for analysis included the effects of collection, pair (collection), diet, and diet × collection interaction.

Experiments 2, 3, and 4 utilized a randomized complete block design. The treatment structure was a 2-way treatment classification (2 × 2 factorial) with 6 replicates per treatment, and the experimental unit was the pig. The ANOVA was also conducted using the GLM procedure of SAS. The model included the effects of collection, diet, the diet × collection interaction, and replicate (collection). Several single degree of freedom preplanned comparisons were performed. Comparisons evaluated the basal diet vs. each additive (VIR or PHY).

The error term reported is the SEM, except for Exp. 2, in which unequal numbers of observations occurred. In that case, the root mean square error is reported, which can be converted into SEM by dividing its value by the square root of the number of observations associated with each specific mean. The α level used for determination of statistical significance was 0.05, with ≤0.10 used to declare a tendency for significance.

After analysis of the individual experiments, all observations from pigs fed the basal and the basal + VIR diets were pooled for a more critical analysis. First, an ANOVA using the GLM procedure of SAS was conducted. The model included the effects of experiment, diet, the diet × experiment interaction, and replicate (experiment). The α level used for determination of statistical significance was 0.05, with ≤0.10 used to declare a tendency for significance. Secondly, the absolute P digestibility and retention response to VIR across all experimental situations within each experiment was plotted relative to the P digestibility and retention responses of the respective control diets (either with or without PHY). The linear regression equations and associated correlation coefficients were computed for the digestibility and retention responses using the treadline formatting functions in Excel (Microsoft, Redmond, WA).

Animals, Housing Conditions, and Dietary Treatment for the Gut Microbiology Experiment

A total of 32 crossbred pigs [24 gilts and 8 barrows; Hampshire × (Yorkshire × Landrace)] with a mean BW of 29.1 × 0.50 kg were used in a 16-wk experiment to evaluate the long-term effects of VIR feeding on gut microbiological profile. Pigs of a common sire were allocated to 1 of 4 dietary treatments, and same-sex pigs were penned by pairs, resulting in 3 replications of females and 1 replication of males. The experiment was conducted in a temperature-controlled room using a total of 16 pens (1.22 × 2.44 m). Each pen was equipped with a single-hole, stainless steel self-feeder and 2 nipple waterers for ad libitum access to feed and water. The diets were fed in meal form.

Four experimental diets were based on corn and SBM, with or without VIR supplementation and with or without a partial deletion of feed-grade dicalcium phosphate (**DICAL**). Diet 1 was a corn-SBM diet with enough DICAL added to meet all NRC (1998) nutrient requirement estimates, including P. Diet 2 was the same as diet 1 but amended with 11 mg/kg of VIR. Diets 3 and 4 met the NRC (1998) nutrient requirement estimates, except for P. Both diets were made slightly P-deficient by deleting 0.15% DICAL. Diet 4 was amended with VIR (11 mg/kg). The treatments were thus arranged as a 2 × 2 factorial arrangement. A similar ratio of calculated dietary Ca:available P among diets was formulated for diets within each stage of growth (growing, 0.60:0.23; developing, 0.50:0.19; and finishing, 0.45:0.15).

Sampling, Laboratory Analysis, and Calculations for the Gut Microbiology Experiment

At the end of the experiment, pigs were humanely killed by exsanguination after electrical stunning. Ileal samples were taken immediately after slaughtering each pig to assess the bacterial profile and digesta pH. Samples consisted of the content from a 20-cm section of the distal ileum. Sampling involved tying both ends of the ileal section with a sterilized cotton thread, cutting the ileum portion, and transporting it on ice to the laboratory for pH determination and bacteria culturing. The quantification of phytate-utilizing bacterial populations, as colony-forming units per gram of ileal contents, was conducted according to the procedure described by Bae et al. (1999).

Experimental Design and Statistical Analysis for the Gut Microbiology Experiment

The treatment structure consisted of a 2 × 2 factorial arrangement with 4 replicate pens/treatment (pen was the experimental unit). The ANOVA was conducted using the GLM procedure of SAS. The statistical model included the effects of each factor, the interaction, and replication. The α level used for determination of statistical significance was 0.05, with ≤0.10 used to declare a tendency for significance.

RESULTS AND DISCUSSION

In general, pigs were in good health and condition during the experiments. All pigs in Exp. 1, 3, and 4 successfully completed the collections. All pigs in Exp. 2 completed the collections, but 1 pig did not gain BW. An important condition in any digestion or balance experiment is that the animals be in a positive balance of nutrients; otherwise, results could be biased by the greater tissue catabolism expected for animals losing BW. Therefore, the pig in Exp. 2 that did not gain BW was not included in the analysis.

Samples of the experimental diets were analyzed for VIR and PHY concentrations by the companies marketing the products. As expected, VIR was not detected in diets not amended with the antibiotic, whereas the other diets, with the exception of diet 4 in Exp. 3, were close to 11 mg/kg (within 25%, Table 2). However, the assays for PHY concentration were different than expected, particularly for diets 3 and 4 in Exp. 2. It is not clear whether the differences reflect an issue with the blending of the diets or with the laboratory analysis. In Exp. 2, both were greater than their target levels and should have been at levels sufficient to maximize the PHY response; therefore, those results were retained. In Exp. 3, the assays for diets 3 and 4 were considered potentially problematic, because differences in results at these relatively low levels of PHY could cause important differences in digestibility due to the enzyme (Cromwell et al., 1995), possibly obscuring potential effects of VIR on the remaining substrate. For this reason, the results for diets 3 and 4 were disregarded for Exp. 3. In Exp. 4, the assay results were closer to those planned, and deviations across diets were deemed to be normal laboratory variation, because diet mixing practices obviated potential mixing mistakes.

Exp. 1

The addition of VIR (11 mg/kg) improved the apparent digestibility of several nutrients (Table 3). Dry matter digestibility increased by 0.94 of a percentage point ($P = 0.05$). A similar improvement (0.84%, $P = 0.06$) was observed for energy digestibility. Nitrogen digestibility was numerically but not statistically improved. In this experiment, the greatest improvement in digestibility was observed for P (8.44%, $P < 0.001$), followed by Ca (5.81%, $P = 0.003$), Zn, and Mg (3.9 and 3.1%, respectively, $P = 0.02$; data not shown). The observed improvement of 8.4 percentage points in P digestibility in Exp. 1 is equivalent to a release of 0.031% additional P in the diet calculated to contain 0.37% total P.

No reports of increased P digestibility in pigs due to VIR or any other antibiotic were found in the literature, except for the experiment by Ravindran et al. (1984),

Table 3. Digestibility (apparent) and retention (as % of absorption) of nutrients in Exp. 1

Item	Treatment[1]		SEM	*P*-value
	Basal	VIR		
DM digestibility, %	88.99	89.93	0.29	0.05
Energy digestibility, %	88.28	89.12	0.27	0.06
P				
Intake, g/d	8.49	8.65	0.09	0.23
Total excreted, g/d	6.11	5.49	0.09	0.001
Absorption, g/d	2.59	3.35	0.12	0.002
Retention, g/d	2.38	3.16	0.12	0.001
Digestibility, %	30.37	38.81	1.11	<0.001
Retention, %	91.90	94.26	0.51	0.01
N				
Intake, g/d	57.5	58.7	0.61	0.22
Total excreted, g/d	28.5	27.7	0.80	0.53
Absorption, g/d	51.0	52.5	0.66	0.16
Retention, g/d	29.1	31.0	1.08	0.24
Digestibility, %	88.69	89.35	0.35	0.21
Retention, %	57.25	59.86	1.86	0.35
Ca				
Digestibility, %	51.51	57.32	0.96	0.003
Retention, %	66.07	70.24	3.07	0.37

[1]Each mean represents 10 individually penned pigs. VIR = 11 mg/kg of virginiamycin.

who used lighter pigs (35 kg of BW) in 3 balance trials to test the effects of the same level of VIR (11 mg/kg) on nutrient digestibility, mineral absorption, retention, and rate of passage in diets supplemented with P. In a 2 × 2 factorial arrangement, they supplemented VIR to a low-fiber corn-SBM meal diet (NDF: 13.5%) and to a high-fiber corn-SBM-oats diet (NDF: 20.2%) to determine if VIR would decrease the greater rate of passage expected for the high-fiber diet, thus improving nutrient utilization. In agreement with previous reports on the effects of fiber, the high-fiber diet decreased DM, energy, CP, and ash digestibility. Interestingly, it was also observed that VIR increased P digestibility in the high-fiber diet (57.7 vs. 63.0%), although it did not affect P digestibility in the low-fiber diet (55.2 vs. 55.4%). Similarly, VIR increased Ca digestibility in the high-fiber diet (56.5 vs. 66.1%) but not in the low-fiber diet (63.5 vs. 62.0%). Phosphorus and Ca retention as a percentage of absorption were also increased by VIR in the high-fiber diet (53.7 vs. 58.3% and 54.4 vs. 64.5%, respectively) but not in the low-fiber diet. Although VIR slowed the rate of passage in both diets (from 20.6 to 26.7 h), the improvement in P digestibility and retention was only observed in the high-fiber diet.

Although Ravindran et al. (1984) did not observe any effect of VIR on P digestibility in a corn-SBM diet, our study did show an improvement. The reason for the difference could be that their diets had more available P (it was supplemented with 0.90% defluorinated phosphate), whereas our diet was P-deficient, so the 2 results may not be completely comparable.

In Exp. 1, besides the improvement in P digestibility, VIR increased P retained as a percentage of absorption ($P = 0.01$). Among all the nutrients measured, P was the only one that showed an improvement in retention (as a percentage of absorbed) with VIR supplementation.

According to the apparent retention observed (calculated as intake minus fecal and urinary losses), VIR-treated pigs retained 0.78 more grams of P per day (3.9 g total for the 5 d) as compared with the controls. With consideration to effects of waste excretion, VIR reduced N and P excretion by 2.8% (from 28.5 to 27.7 g/d; $P = 0.53$) and 10.1% (from 6.11 to 5.49 g/d; $P = 0.001$), respectively.

Exp. 2, 3, and 4

VIR Effects. In these experiments, a main effect of VIR on P digestibility was not observed. Nevertheless, a tendency for a VIR × PHY interaction was observed in Exp. 2 (Table 4; $P = 0.06$). Given that differences due to VIR were observed in Exp. 1, single degree of freedom comparisons were made between diets 1 and 2 in Exp. 2, 3, and 4. The comparisons for differences in P digestibility numerically favored VIR in all experiments, but the *P*-value differed: Exp. 2 (34.61 vs. 39.25%, $P = 0.027$), Exp. 3 (32.45 vs. 37.66%, $P = 0.063$), and Exp. 4 (33.40 vs. 35.18%, $P = 0.44$).

In contrast to Exp. 1, in which the digestibilities of DM, energy, P, Ca, Mg, and Zn were significantly increased by the inclusion of VIR, statistically significant differences were less prominent for these nutrients in the other 3 experiments. Tendencies for main effect improvements were observed only for N digestibility in Exp. 2 ($P = 0.10$) and also for Zn and Cu digestibility in Exp. 4 ($P < 0.10$; data not shown).

Concerning P retention, expressed as a percentage of absorbed P, there was a main effect of VIR in Exp. 2 (Table 4; $P = 0.03$) and numerical differences in Exp. 3 and 4 (Tables 5 and 6). Virginiamycin also tended to increase P retention, expressed as a percentage of P intake (data not shown), in Exp. 3 ($P = 0.08$), and numerical differences were observed in Exp. 2 and 4. These differences in statistical response could suggest an equivocal response to the use of VIR. However, the digestibility and retention data can be pooled across experiments to obtain greater statistical power. When that is done (Table 7), the improvements in P and Ca responses are unequivocal. Across experiments, the average improvement in P digestibility by amending the basal diet with VIR was 5.0% (range: 1.78 to 8.44%; $P < 0.001$), whereas the average improvement in P retention (as a percentage of absorption) was 1.0% (range: 0.07 to 2.36%; $P = 0.004$). Averaging values from the 4 experiments, the decrease in total P excreted due to VIR amendment was 4.9% (data not shown; $P = 0.006$).

Phytase Effects. The addition of 750 PU/kg of diet had a strong positive effect on the digestibility of several minerals, particularly P and Ca (Table 4). Results from Exp. 2 indicate that this level of PHY inclusion increased P digestibility 78.9% (from 34.6 to 61.9%) for

Table 4. Digestibility (apparent) and retention (as % of absorption) of nutrients (least squares means) in Exp. 2

	Treatment[1]					*P*-value[2]		
	Basal	VIR	PHY	VIR + PHY				
Item	1	2	3	4	RMSE[3]	VIR	PHY	VIR × PHY
DM digestibility, %	91.61	92.14	91.82	92.28	0.74	0.15	0.59	0.91
Energy digestibility, %	90.85	91.38	90.52	91.04	0.74	0.13	0.32	0.99
P								
Intake, g/d	7.24	7.57	7.72	7.61	0.35	0.46	0.11	0.17
Total excreted, g/d	4.84	4.73	3.07	3.08	0.31	0.71	<0.001	0.65
Absorption, g/d	2.52	2.94	4.76	4.63	0.31	0.28	<0.001	0.06
Retention, g/d	2.39	2.84	4.65	4.53	0.30	0.23	<0.001	0.05
Digestibility, %	34.61	39.25	61.91	60.98	3.15	0.19	<0.001	0.06
Retention, %	95.17	96.53	97.66	97.96	0.76	0.03	<0.001	0.13
N								
Intake, g/d	55.02	57.56	58.66	57.89	2.70	0.46	0.11	0.18
Total excreted, g/d	29.36	31.89	30.77	29.64	3.27	0.62	0.77	0.21
Absorption, g/d	49.45	52.29	52.52	52.26	2.37	0.23	0.16	0.15
Retention, g/d	25.66	25.67	27.88	28.25	2.59	0.87	0.05	0.88
Digestibility, %	89.83	90.81	89.43	90.22	1.17	0.10	0.35	0.85
Retention, %	51.43	49.65	52.77	54.44	4.92	0.98	0.17	0.43
Ca								
Digestibility, %	59.49	61.69	71.27	68.74	3.09	0.90	<0.001	0.10
Retention, %	77.82	72.47	81.48	84.17	5.62	0.59	0.01	0.12

[1]VIR = basal + 11 mg/kg of virginiamycin; PHY = basal + 750 phytase units/kg of diet; VIR + PHY = basal + 11 mg/kg of virginiamycin + 750 phytase units/kg of diet.
[2]VIR = virginiamycin effect; PHY = phytase effect; VIR × PHY = virginiamycin × phytase interaction.
[3]RMSE = root mean square error (number of pigs = 6, 6, 5, and 6 pigs for dietary treatments 1, 2, 3, and 4, respectively).

the basal diet and 55.4% (from 39.3 to 61.0%) for the VIR-added diet ($P < 0.001$). As expected, a smaller effect on P digestibility was observed when PHY level was reduced to 300 PU in Exp. 4, which agrees with earlier reports of linear responses to different levels of PHY (Lei et al., 1993; Veum et al., 1994; Cromwell et al., 1995).

Table 5. Digestibility (apparent) and retention (as % of absorption) of nutrients in Exp. 3

	Treatment[1]			
Item	Basal	VIR	SEM	*P*-value
DM digestibility, %	90.54	90.32	0.31	0.64
Energy digestibility, %	90.41	89.99	0.34	0.43
P				
Intake, g/d	6.03	6.28	0.10	0.15
Total excreted, g/d	4.08	3.91	0.08	0.21
Absorption, g/d	1.98	2.39	0.13	0.09
Retention, g/d	1.96	2.37	0.13	0.09
Digestibility, %	32.45	37.66	1.60	0.08
Retention, %	98.81	99.10	0.18	0.32
N				
Intake, g/d	46.04	47.95	0.75	0.15
Total excreted, g/d	26.69	26.21	0.41	0.45
Absorption, g/d	41.35	42.95	0.74	0.20
Retention, g/d	19.34	21.73	0.89	0.13
Digestibility, %	89.63	89.43	0.62	0.83
Retention, %	47.29	50.83	1.62	0.20
Ca				
Digestibility, %	57.93	61.22	1.79	0.26
Retention, %	50.33	61.48	2.64	0.04

[1]Each mean represents 6 individually penned pigs; VIR = basal + 11 mg/kg of virginiamycin.

In Exp. 2, no effect of PHY on DM, energy, or N digestibility was observed. But, PHY did improve Ca digestibility in both Exp. 2 and 4 (Tables 4 and 6, respectively; $P < 0.001$), which is in agreement with other reports (Lei et al., 1993; Pallauf et al., 1994; Jongbloed et al., 1999b).

In Exp. 2 (Table 4), the tendency for an interaction between PHY and VIR for P and Ca digestibility ($P < 0.10$), along with the lack of significant difference between diets 3 and 4, indicated that the level of inclusion of PHY was probably high enough to release a large proportion of the phytate P present in the diet, not leaving any room for further VIR effect. This observation motivated reduction of the PHY level in Exp. 3 and 4 to facilitate detection of any possible additive effects of VIR.

As expected, a smaller response in digestibility was observed when the level of PHY was lowered in Exp. 4 (Table 6). It is known that there is a dose-response relationship between the level of PHY and the apparent total tract P digestibility in pigs fed corn-SBM diets (Cromwell et al., 1995; Harper et al., 1997; Jongbloed et al., 1999a). In Exp. 2, comparisons between diets 1 and 3 showed that PHY increased P digestibility 79% (from 34.6 to 61.9%, $P < 0.01$; Table 4), but it was only 41% (from 33.4 to 47.2%, $P < 0.01$) in Exp. 4 (Table 6). Phosphorus retention (g/d) was also increased by PHY

Table 6. Digestibility (apparent) and retention (as % of absorption) of nutrients in Exp. 4 (least squares means)

	Treatment[1]							
	Basal	VIR	PHY	VIR + PHY		*P*-value[2]		
Item	1	2	3	4	SEM	VIR	PHY	VIR × PHY
DM digestibility, %	90.08	89.69	90.01	90.33	0.27	0.91	0.31	0.21
Energy digestibility, %	89.52	88.96	88.95	89.23	0.30	0.65	0.64	0.19
P								
Intake, g/d	6.28	6.28	6.34	6.47	0.21	0.76	0.56	0.75
Total excreted, g/d	4.28	4.16	3.42	3.28	0.20	0.55	0.001	0.97
Absorption, g/d	2.07	2.19	2.99	3.26	0.11	0.100	<0.001	0.49
Retention, g/d	2.00	2.12	2.92	3.19	0.11	0.10	<0.001	0.50
Digestibility, %	33.40	35.18	47.18	50.31	1.56	0.14	<0.001	0.67
Retention, %	96.54	96.61	97.59	97.71	0.23	0.68	0.001	0.90
N								
Intake, g/d	45.75	45.74	46.17	47.14	1.53	0.76	0.56	0.75
Total excreted, g/d	20.46	20.58	19.96	19.82	1.28	0.99	0.63	0.92
Absorption, g/d	41.08	41.01	41.38	42.38	1.32	0.73	0.54	0.69
Retention, g/d	25.29	25.15	26.22	27.32	1.16	0.68	0.21	0.60
Digestibility, %	89.74	89.84	89.52	89.92	0.49	0.61	0.89	0.76
Retention, %	61.25	61.83	63.63	64.27	2.10	0.78	0.27	0.99
Ca								
Digestibility, %	51.02	52.97	60.91	63.20	1.54	0.19	<0.001	0.91
Retention, %	55.26	53.13	67.39	68.33	2.60	0.82	<0.001	0.57

[1]Each mean represents 6 individually penned pigs. VIR = basal + 11 mg/kg of virginiamycin; PHY = basal + 300 phytase units/kg of diet; VIR + PHY = basal + 11 mg/kg of virginiamycin + 300 phytase units/kg of diet.
[2]VIR = virginiamycin effect; PHY = phytase effect; VIR × PHY = virginiamycin × phytase interaction.

additions ($P < 0.001$); the percentage increases resemble the changes observed in P digestibility.

Reflecting the increase in digestibility and retention observed with the PHY amendments, total P excretion was notably reduced by PHY. In Exp. 2 (Table 4), PHY decreased ($P < 0.001$) total P excretion of the basal diet by 36.6% (from 4.8 to 3.1 g/d) and by 34.9% (from 4.7 to 3.1 g/d) in the VIR-added diet. Smaller decreases in P excretion were observed when the PHY level was reduced to 300 PU in Exp. 4. In Exp. 4 (Table 6), P excretion decreased ($P = 0.001$) 20.1% for the basal diet (from 4.3 to 3.4 g/d) and 21.2% for the VIR-added diet (from 4.2 to 3.3 g/d).

Table 7. Digestibility (apparent) and retention (as % of absorption) of nutrients pooled across Exp. 1 to 4 (least squares means)

	Treatment[1]			
Item	Basal	VIR	SEM	*P*-value
No. of barrows	28	28		
DM				
Digestibility, %	90.30	90.52	0.14	0.30
Energy				
Digestibility, %	89.76	89.86	0.15	0.64
P				
Digestibility, %	32.71	37.72	0.71	<0.001
Retention, %	95.60	96.63	0.22	0.004
N				
Digestibility, %	89.47	89.85	0.23	0.25
Retention, %	54.30	55.54	1.28	0.50
Ca				
Digestibility, %	54.99	58.30	0.69	0.002
Retention, %	62.37	64.33	1.63	0.40

[1]Each mean represents 28 individually penned pigs. VIR = basal + 11 mg/kg of virginiamycin.

From the results of the 4 experiments (Table 7), it was evident that VIR amendment improved apparent P digestibility by approximately 5%. In addition, the PHY amendment results confirmed the positive effects of this enzyme on digestibility, retention, and excretion. But, there were differences in the VIR response across experiment and level of PHY supplementation. The relative P response across all situations is best assessed in the manner presented in Figure 1, in which the P responses for VIR are plotted against the P digestibility of their respective controls (either with or without PHY). It is evident that the magnitude of improvement is dependent on the digestibility of the control; specifically, the magnitude of increase in P digestibility due to VIR tended to be inversely related to the P digestibility of the control diet. It should be noted that there is a VIR digestibility response in diets containing PHY if PHY used is not the level that can maximize P digestibility. That is, there is potential for an additivity of response to the 2 factors evaluated in these experiments

Lindblad et al. (1954) arrived at a similar conclusion for chicks and poults fed Ca- and P-deficient diets supplemented with aureomycin. They showed that in the absence of the antibiotic, maximum BW gain was obtained with a diet containing 1.0% Ca and 0.6% inor-

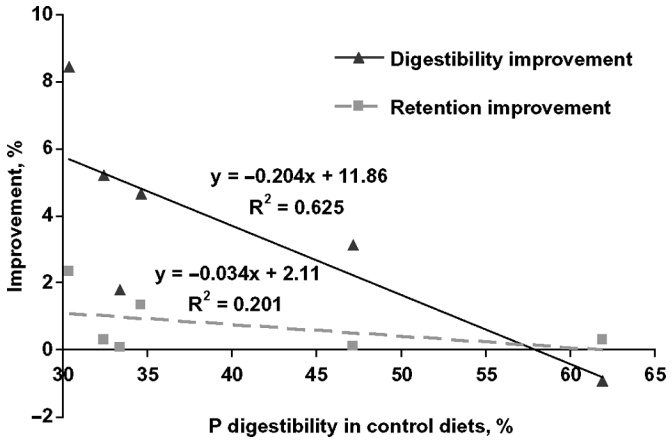

Figure 1. Percentage unit change in P digestibility and retention (% of absorption) in response to virginiamycin supplementation in Exp. 1, 2, 3, and 4 based on the digestibility level of the respective control diets.

ganic P. However, in the presence of aureomycin, maximum gain and efficiency of feed utilization resulted when P was decreased to 0.4%. Increasing P above 0.4% in the diet containing antibiotic did not increase the gain. The researchers concluded that the more inadequate the Ca and P in the diet, the greater the percentage increase in BW gain was achieved from the antibiotic.

The inverse relationship observed between the level of improvement in P digestibility and the digestibility level of the basal diet is in agreement with the conclusions of Braude and Johnson (1953) relative to effects of antibiotic on growth. Those researchers summarized many experiments with different antibiotics to conclude that the relative improvement in growth resulting from antibiotic amendments was inversely related to the growth rate of the control pigs.

Gut Microbiology Experiment

The long-term feeding of VIR in both the control diet and the diet with a marginally reduced P level resulted in a change in ileal microbial profile (Table 8). Phytate-utilizing bacteria were the intestinal organisms of greatest interest. A positive increment in the number of these bacteria was observed in both the normal and P-deleted diets when VIR was added, although the differences were not statistically significant (P = 0.13). This numerical difference represents logarithm increments of 12.4 and 17.2% over the respective controls. No literature reports were found on the effects of antibiotics on phytate-utilizing bacteria. The addition of VIR also tended to affect lactobacilli populations (main effect, P = 0.11; interaction, P = 0.02). Virginiamycin strongly decreased lactobacilli in the normal-P diet but did not affect this bacterial population in the P-deleted diet. The observed results on changes in lactobacilli numbers in the normal-P diet amended with VIR agree with the results of Collier et al. (2003), who reported a decrease in lactobacilli counts in pigs fed a rotating sequence of antibiotics, including VIR. Other researchers have shown decreasing numbers of lactobacilli in the feces and incubated ileal contents of pigs fed different antibiotics (Andersen, 1954; Vervaeke et al., 1979). Because lactobacilli are abundant populations of acid-producing bacteria, an increase in ileal pH would also be expected to coincide with a lactobacilli population decrease. Nevertheless, no difference in pH was observed in this experiment. Collier et al. (2003) did not provide data on pH changes, whereas Vervaeke et al. (1979) reported a decrease in lactobacilli numbers that was associated with a corresponding increase in pH for ileal contents of pigs fed VIR. Due to known differences in populations of dominant bacterial species in the different segments of the small intestine (Fewins et al., 1957), and taking into account that the major site of

Table 8. Ileal bacterial counts ($\log_{10}$ cfu/g) and ileal chyme pH in the gut microbiology experiment

	Diet[1]				*P*-value			
Item	1	2	3	4	SEM	VIR	DICAL	VIR × DICAL
VIR[2]	–	+	–	+				
DICAL deletion[3]	–	–	+	+				
Response								
Phytate utilizing	7.35	8.26	7.03	8.24	0.62	0.13	0.79	0.82
Lactobacilli	9.58	7.84	8.25	8.69	0.37	0.11	0.53	0.02
Total coliforms	8.11	8.34	7.93	8.34	0.60	0.60	0.88	0.88
Total anaerobes	10.00	8.61	8.95	9.38	0.68	0.50	0.84	0.21
Escherichia coli	7.63	8.04	7.62	7.78	0.60	0.65	0.83	0.84
Bifido	9.70	8.90	9.02	9.24	0.54	0.61	0.72	0.37
pH	6.9	6.9	6.8	6.8	0.11	0.89	0.32	0.58

[1]Means represent 4 pens, averaging both pigs per pen (except for diet 3, in which a pneumonic pig was removed from the study).
[2]VIR = virginiamycin, 11 mg/kg.
[3]DICAL = dicalcium phosphate. In diets in which DICAL was reduced, the amount was equivalent to the amount that would provide 0.03% of total P.

Ca and P absorption is the jejunum (Crenshaw, 2001), it might be important for future research to also assess microbial populations and pH differences at the jejunum.

In conclusion, the antibiotic VIR improves both Ca and P digestibility in pigs. The increase in digestibility is not as great as that provided by PHY, but because the potential mechanism of action (altered microbial populations with VIR) differs from that of PHY (direct addition of an enzyme), there can be a degree of additivity in P digestibility improvements when both products are used.

LITERATURE CITED

Andersen, H. E. 1954. Change in the intestinal flora produced by peroral administration of penicillin in normal swine. Nord. Vet. Med. 6:622–642.

AOAC. 1990. Official Methods of Analysis. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 1995a. Official Methods of Analysis. 16th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 1995b. Official Methods of Analysis. 16th ed. 3rd rev. Suppl. Mar. 1997. Assoc. Off. Anal. Chem., Arlington, VA.

Bae, H. D., L. J. Yanke, K. J. Cheng, and L. B. Selinger. 1999. A novel staining method for detecting phytase activity. J. Microbiol. Methods 39:17–22.

Braude, R., and B. C. Johnson. 1953. Effect of aureomycin on nitrogen and water metabolism in growing pigs. J. Nutr. 49:505–512.

Brown, W. O. 1957. The effect of dietary penicillin on calcium and nitrogen retention in chicks on a low mineral diet. J. Sci. Food Agric. 8:279–282.

Buresh, R. E., R. D. Miles, and R. H. Harms. 1985. Influence of virginiamycin on phosphorus utilization by broiler chicks. Poult. Sci. 64:757–758.

Collier, C. T., M. R. Smiricky-Tjardes, D. M. Albin, J. E. Wubben, V. M. Gabert, B. Deplancke, D. Bane, D. B. Anderson, and H. R. Gaskins. 2003. Molecular ecological analysis of porcine ileal microbiota responses to antimicrobial growth promoters. J. Anim. Sci. 81:3035–3045.

Crenshaw, T. D. 2001. Calcium, phosphorus, vitamin D, and vitamin K in swine nutrition. Page 187–212 in Swine Nutrition. 2nd ed. A. J. Lewis and L. L. Southern, ed. CRC Press LLC, Boca Raton, FL.

Cromwell, G. L., R. D. Coffey, G. R. Parker, H. J. Monegue, and J. H. Randolph. 1995. Efficacy of a recombinant-derived phytase in improving the bioavailability of phosphorus in corn-soybean meal diets for pigs. J. Anim. Sci. 73:2000–2008.

Dierick, N. A., I. J. Vervaeke, J. A. Decuypere, and H. K. Henderickx. 1986. Influence of the gut flora and of some growth-promoting feed additives on nitrogen metabolism in pigs. II. Studies in vivo. Livest. Prod. Sci. 14:177–193.

Fewins, B. G., L. G. M. Newland, and C. A. E. Briggs. 1957. The normal intestinal flora of the pig. Qualitative studies of lactobacilli and streptococci. J. Appl. Bacteriol. 20:234–242.

Harper, A. F., E. T. Kornegay, and T. C. Schell. 1997. Phytase supplementation of low-phosphorus growing-finishing pig diets improves performance, phosphorus digestibility, and bone mineralization and reduces phosphorus excretion. J. Anim. Sci. 75:3174–3186.

Jongbloed, A. W., P. A. Kemme, and Z. Mroz. 1999a. Effectiveness of Natuphos phytase in improving the bioavailabilities of phosphorus and other nutrients for growing-finishing pigs. Page 259–274 in Phytase in Animal Nutrition and Waste Management. 2nd ed. M. B. Coelho and E. T. Kornegay, ed. BASF Ref. Man. DC9601. BASF Corp., Mount Olive, NJ.

Jongbloed, A. W., P. A. Kemme, and Z. Mroz. 1999b. Influence of dietary calcium levels on the effectiveness of Natuphos phytase for swine. Page 393–400 in Phytase in Animal Nutrition and Waste Management. 2nd ed. M. B. Coelho and E. T. Kornegay, ed. BASF Ref. Man. DC9601. BASF Corp., Mount Olive, NJ.

Lei, X. G., P. K. Ku, E. R. Miller, and M. T. Yokoyama. 1993. Supplementing corn-soybean meal diets with microbial phytase linearly improves phytate phosphorus utilization by weanling pigs. J. Anim. Sci. 71:3359–3367.

Lindblad, G. S., S. J. Slinger, and I. Motzok. 1954. Effect of aureomycin on the calcium and phosphorus requirements of chicks and poults. Poult. Sci. 33:482–491.

NRC. 1998. Nutrient Requirements of Swine. 10th ed. Natl. Acad. Press, Washington, DC.

Pallauf, J., G. Rimbach, S. Pippig, B. Schindler, and E. Most. 1994. Effect of phytase supplementation to a phytate-rich diet based on wheat, barley and soya on the bioavailability of dietary phosphorus, calcium, magnesium, zinc and protein in piglets. Agribiol. Res. 47:39–48.

Ravindran, V., E. T. Kornegay, and K. E. Webb Jr. 1984. Effects of fiber and virginiamycin on nutrient absorption, nutrient retention and rate of passage in growing swine. J. Anim. Sci. 59:400–408.

Schutte, J. B., G. M. Beelen, and E. Boselli. 1994. Effect of virginiamycin on apparent ileal digestibility of dietary nutrients in adult roosters. Pages 443–444 in Proc. 9th Eur. Poult. Conf., Glasgow, UK.

Vervaeke, I. J., J. A. Decuypere, N. A. Dierick, and H. K. Henderickx. 1979. Quantitative in vitro evaluation of the energy metabolism influenced by virginiamycin and spiramycin used as growth promoters in pig nutrition. J. Anim. Sci. 49:846–856.

Veum, T. L., J. Liu, D. W. Bollinger, K. Zyla, and D. R. Ledoux. 1994. Use of a microbial phytase in a canola-grain sorghum diet to increase phosphorus utilization by growing pigs. J. Anim. Sci. 72(Suppl. 1):218. (Abstr.)