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Raw potato starch in weaned pig diets and its influence on postweaning scours and the molecular microbial ecology of the digestive tract

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ABSTRACT: We evaluated the effect of raw potato starch (RPS) on growth performance, postweaning diarrhea, and gastrointestinal microbial populations in weaned piglets. Eighty-four piglets were weaned at 17 ± 2 d of age with an average BW of 6.0 ± 0.9 kg. Pigs were blocked by BW and assigned to 1 of 4 diets in a randomized complete block design with 7 replicate pens per diet and 3 pigs per pen. Treatments were 1) a positive control (PC) containing an antibiotic, 2) a negative control (NC) with no RPS and no antibiotic, 3) NC + 7% RPS (7% RPS), and 4) NC + 14% RPS (14% RPS). Diets were corn-wheat-soybean meal-based and formulated to meet NRC (1998) recommendations. The ADG, ADFI, and G:F ratio were determined weekly. Fecal consistency (FC) scoring was determined daily. After wk 3, 1 pig with a BW closest to the pen mean was killed to evaluate ileal and colonic mucosal-attached Escherichia coli and lactic acid bacteria, as well as digesta pH, VFA, and ammonia N concentrations. The DNA was extracted from ileum and colon digesta and used for molecular microbial evaluations using terminal-RFLP analysis of 16S rDNA genes. The ADG for wk 1 was greater (P < 0.01) for the PC diet, but diet had no effect on ADG during wk 3. The ADFI did not differ among treatments during the first 2 wk, and ADFI was least for 7% RPS diet during wk 3. The NC diet had a greater (P < 0.05) FC score during wk 1 than other treatments, but diet had no effect on FC score during wk 2 and 3. Diets had no effect on the colon lactic acid bacterial counts; however, the PC diet had decreased (P < 0.05) colon E. coli counts than other treatments. Ileum and colon digesta pH and total VFA concentrations did not differ among treatments. Pigs fed with 7 and 14% RPS diets had greater (P < 0.05) ileum ammonia N concentration compared with pigs fed with other diets. There was more diarrhea (P < 0.05) in the 14% than the 7% RPS and control treatments at d 21. This difference correlated with a decline (P < 0.05) in microbial diversity in the colon. We concluded that 7% RPS can be used to prevent postweaning diarrhea in weaned piglets, but there are no effects on growth performance.

Key words: antibiotic, diarrhea, Escherichia coli, microbial ecology, raw potato starch

INTRODUCTION

Weaned pigs often develop diarrhea from microbial infections or nutritional imbalances. Pigs become dehydrated, lose BW, and are generally morbid, and the most effective way of preventing the problem is by including subtherapeutic antibiotics in the feed. The emergence of antibiotic-resistant bacteria in human medicine and the potential relationship between subtherapeutic inclusions of in-feed antibiotics has resulted in pressure to remove antibiotics from swine diets (Stein and Kil, 2006). However, the removal of feed antibiotics can lead to an increase in the use of therapeutic antibiotics in swine production because of an increase in digestive and respiratory infections (Bengtsson and Wierup, 2006; Jensen, 2006). Thus, simply removing antibiotics from feed is not sufficient, and feed additives that have antimicrobial activity, but are not antibiotics, are needed (Pettigrew, 2006).

One alternative is the use of prebiotics like resistant starch (RS; Englyst et al., 1992). Resistant starch refers to starch, plus its digestion products that are not absorbed in the small intestine and pass to the large
bowel and beneficially modify gut microbial populations (Englyst et al., 1992). Resistant starch is categorized into (a) physically inaccessible starches (RS1), (b) resistant granules (RS2), (c) retrograde starch (RS3), and (d) chemically modified starch (RS4). In this study, we evaluated the effects of raw potato starch (RPS; RS2 resistant starch) in weaned pigs and its potential to prevent diarrhea. We also evaluated the effects of RPS on gut microbial populations using terminal-RFLP analysis (T-RFLP) of bacterial 16S rDNA genes.

MATERIALS AND METHODS

The experimental protocol was reviewed and approved by the University of Manitoba Animal Care Committee. Pigs were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

Animals, Housing, and Experimental Design

A total of 84 piglets weaned at 17 ± 1 d were obtained from the Glenlea swine research farm of the University of Manitoba and assigned to treatments in a randomized complete block design. Pigs were weighed and assigned to outcome groups on the basis of BW and sex, and randomly allocated to 4 treatments, consisting of 3 pigs per pen, and 7 replicates. Each pen had a plastic-covered expanded metal floor, a stainless-steel feeder, and a low-pressure nipple drinker. Pigs had unlimited access to feed and water throughout the 3-wk study. Body weight and feed disappearance were monitored weekly, and the results were used to calculate ADG, ADFI, and G:F. Room temperature was maintained at 29 ± 1°C throughout the study.

Experimental Diets

Diets included 1) a positive control containing an antibiotic (Aureo SP250, Alpharma Inc., Fort Lee, NJ; PC); 2) a negative control containing no RPS or antibiotics (NC); 3) negative control + 7% RPS (7% RPS); and 4) a negative control + 14% RPS (14% RPS). All diets contained 10% pea protein isolate (PPI), which was included as a nutritional stress treatment to induce diarrhea (Owusu-Asiedu et al., 2003a,b). Experimental diets were based on corn, wheat, and soybean meal and formulated to meet NRC (1998) nutrient requirements for piglets weighing 7 to 12 kg (Table 1). Diets were mixed 1 wk before the start of the experiment using the same batch of ingredients. Diets were provided ad libitum in a mash form. The severity of diarrhea was measured using a fecal consistency (FC) scoring system (Marquardt et al., 1999). Fecal consistency scoring (0, normal; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea) was performed in a blinded fashion by 2 individuals trained to differentiate among feces type. The presence of blood in feces was checked daily.

Tissue and Digesta Sampling

At the end of the study, the pig with a BW closest to the mean pen BW was held under general anesthesia and killed by an intracardiac injection of sodium pentobarbital (50 mg/kg BW). Following euthanasia the abdominal cavity was opened from sternum to pubis to expose the gastrointestinal tract without damaging the wall of the digestive tract. The stomach, small intestine, and large intestine were weighed with and without digesta to determine digesta and empty weights, respectively. The liver was also weighed. The small intestine was stripped free of its mesentery and further divided into 3 sections: 1) the ileum from the ileal-cecal junction to 80-cm anterior to this junction; 2) the duodenum, 80 cm posterior to the gastro-duodenal sphincter; and 3) the jejunum constituted the regions between the ileum and duodenum (Adeola and King, 2006). Digesta samples were taken from the ileum and colon (proximal) for measurement of pH, VFA, and ammonia N concentrations. The pH was determined immediately using a pH meter (AB 15, Fisher Scientific, Pittsburgh, PA), and subsamples (approximately 5 g) from each gut segment were mixed with 5 mL of 0.1 M HCl to stop microbial activity and stored at −25°C until analyzed for VFA and lactic acid, using gas chromatography as described by Erwin et al. (1961). Ammonia N concentration was measured using the indole phenol-blue method (Novozamsky et al., 1974). Starch concentration in the ileum and colon were measured and expressed as % DM (AOAC, 2005).

Enumerations of Adherent Bacteria

Tissues were weighed (10 g), washed vigorously with sterile physiological saline to remove nonattached bacteria, and a blunt knife was used to scrape off the epithelial tissue, which was weighed and subsequently diluted 10-fold with PBS, homogenized, and decimal-diluted. Ten-microliter droplets were pipetted onto chromogenic Escherichia coli/Coliform media (EMB, Oxoid, Nepean, Ontario, Canada) and de Man, Rogosa, and Sharpe media (MRS; Fisher Scientific, Ottawa, Ontario, Canada; 10−1 to 10−3 dilutions) allowed to dry, and then inverted and incubated aerobically at 39 ± 1°C for 24 to 36 h. The numbers of E. coli and lactic acid bacteria were expressed as colony-forming units/gram of mucosa.

Molecular-Based Analysis

The DNA was extracted from the ileum and colon contents using the ZR-DNA fecal Kit (Zymo Research, Orange, CA) as per manufacturer protocol. To check for DNA concentration, a sample was run on a 1%
agarose gel to determine if a high molecular weight band indicative of intact chromosomal DNA was visible. The DNA was measured spectrophotometrically at 260 nm, and all samples were diluted to an equal concentration of 100 µg·mL⁻¹ DNA. Terminal-RFLP analysis was used to assess the changes in microbial composition in the gut (Abdo et al., 2006). Primers 27f (5′-GAAGAGTTTGATCATGGCTCAG-3′) and 1100r (5′-CTGCTGCCTCCCGTAG3′) were used to amplify an informative sequence of the 16S rDNA gene (Lane, 1991). The forward primer was fluorescently labeled (WellRED D4dye, Sigma-Proligo, St. Louis, MO) to allow detection of the fragments by capillary electrophoresis. The PCR reactions were as follows: 1 cycle of 94°C for 5 min, then 36 cycles at 94°C for 1 min; 56°C for 1 min; 72°C for 2 min; and a final extension at 72°C for 5 min. To produce terminal restriction fragments (T-RF), the 27 to 1100 region of 16S DNA was digested using HhaI (10 µL of PCR product, 10 units of HhaI, 1X HhaI buffer, and 20 µg of bovine serum). The mixture was adjusted to a final volume of 20 µL with MilliQ water and the DNA was digested at 37°C for 3 h. The precise length of T-RF amplicons were determined on a CEQ 8800 Genetic Analysis System (Beckman Coulter Inc., Fullerton, CA). Six microliters of fluorescently labeled fragments, 26 µL sample loading solution, and 0.5 µL of a DNA size standard (600 bp for T-RFLP) were mixed and separated.

Bioinformatic Analysis of T-RFLP Data

Microbial community analysis (MiCA, version 3; Department of Biological Sciences, University of Idaho; http://mica.ibest.uidaho.edu/) Web services were used to build a putative reference database of probable T-RF of the gut. For this purpose we incorporated 16S rDNA clone libraries of near complete sequences of gut microorganisms found in human (Eckburg et al., 2005), swine (Leser et al., 2002), mouse (Ley et al., 2005), and ruminants (Nelson et al., 2003; Ozutsumi et al., 2005) into MiCA, which we called the H.Q. database. This greatly facilitated analysis by excluding the T-RF that are unlikely to occur in the gut, because in general only 8 out of 26 recognized phyla and no candidate phyla of note have been found in the digestive tract (Leser et al., 2002; Rappe and Giovannoni, 2003; Eckburg et al., 2005; Ley et al., 2005). The fragment profiles produced by HhaI restriction of the 27 to 1100 product were applied to the H.Q. database in silico so that a reference library for our study could be constructed and exported to the phylogenetic assignment tool (Kent et al., 2003). Concurrently, using T-RFLP data obtained

### Table 1. Composition and nutrient analysis of experimental diets

| Item                          | PC     | NC     | 7% RPS | 14% RPS |
|-------------------------------|--------|--------|--------|---------|
| Ingredient, %                 |        |        |        |         |
| Corn                          | 25.80  | 25.80  | 13.85  | 8.85    |
| Soybean meal, 48% CP          | 10.00  | 10.00  | 11.00  | 13.00   |
| Wheat                         | 36.00  | 36.00  | 36.00  | 34.00   |
| Whey powder                   | 10.00  | 10.00  | 14.00  | 12.00   |
| Limestone                     | 0.75   | 0.75   | 0.75   | 0.75    |
| Dicalcium phosphate           | 1.60   | 1.60   | 1.60   | 1.60    |
| Soybean oil                   | 4.60   | 4.60   | 4.60   | 4.60    |
| L-Lysine-HCl                  | 0.15   | 0.15   | 0.10   | 0.10    |
| Vitamin premix²               | 0.50   | 0.50   | 0.50   | 0.50    |
| Mineral premix³               | 0.50   | 0.50   | 0.50   | 0.50    |
| L-Tryptophan                  | 0.10   | 0.10   | 0.10   | 0.10    |
| PPI⁴                          | 10.00  | 10.00  | 10.00  | 10.00   |
| Potato starch                 | —      | —      | 7.00   | 14.00   |
| ASP250⁵                       | 0.01   | —      | —      | —       |
| Calculated nutrient content   |        |        |        |         |
| GE, kcal/kg                   | 3,982.9| 3,982.9| 4,049.7| 4,094.9 |
| CP, %                         | 21.13  | 21.13  | 21.18  | 21.18   |
| Total lysine, %               | 1.28   | 1.28   | 1.28   | 1.30    |
| Fiber (%)                     | 2.13   | 2.13   | 1.91   | 1.87    |
| Analyzed nutrient content     |        |        |        |         |
| GE, kcal/kg                   | 4,319.3| 4,398.0| 4,228.5| 4,350.2 |
| CP                            | 23.40  | 23.20  | 22.40  | 22.70   |

¹Diets: PC = positive control, diet containing antibiotic; NC = negative control with no potato starch or antibiotic; 7% RPS = negative control + 7% potato starch; 14% RPS = negative control + 14% potato starch.

²Provided per kilogram of diet: 9,000 IU of vitamin A; 1,500 IU of vitamin D₃; 18 mg of vitamin E; 1.5 mg of vitamin K; 250 mg of choline; 30 mg of niacin; 27.5 mg of calcium pantothenate; 9.4 mg of B₂; 2 mg of B₆; 25 µg of B₁₂; 80 µg of biotin; 0.5 mg of folic acid.

³Provided per kilogram of diet: 18 mg of copper; 110 mg of zinc; 0.2 mg of iodine; 110 mg of iron; 50 mg of manganese; and 0.3 mg of selenium.

⁴PPI = pea protein isolate; Carman, Manitoba, Canada.

⁵Aureo SP250: Chlortetracycline, Penicillin (as penicillin G Procaine), Sulfamethazine; Alpharma Inc., Fort Lee, NJ.
from CEQ software (fragment sizes and peak areas), various profiles of interest were developed with reference to treatment. These libraries were entered into the hierarchical browser of the ribosomal database project (RDP-II, Cole et al., 2005) and converted to GenBank format. The resulting libraries were assigned to the library compare tool of RDP-II. The T-RF of the same size were in many cases ambiguous in their assignment of taxonomic rank (Sepehri et al., 2007). To resolve this problem the T-RF with multiple accession numbers were assigned to a taxonomic rank according to phylum, class, order, and family. Data were analyzed using Fisher’s exact test (SAS Inst. Inc., Cary, NC).

Richness and Diversity Indices

A diversity index is a mathematical measure of species diversity in a community. Diversity indices provide information about community composition and take the relative abundances of different species into account. The concept of diversity has 2 facets: richness or the number of taxonomic units, and evenness, or equality in the abundances of each taxonomic units. Incidence-based richness indicators, Chao2, ICE (incidence-based coverage estimator) and MMMean (Michaelis-Menten mean) function, along with Shannon and Simpson diversity indices, were calculated using EstimateS 7.5 (Colwell, 2005). Several estimators were selected because if indices follow the same trend regardless of the calculation method, the results are likely to be robust. An upper abundance limit of 5 was used to determine rare or infrequent species. The order of the samples was randomized 500 times for each run to reduce the effect of sample order. Tukey’s multiple comparison test (SAS Inst. Inc.) was applied to detect significant differences among experimental groups.

Chemical Analysis

Dietary CP was analyzed using a Leco NS 2000 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI). Gross energy was measured using a Parr adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL).

Calculations and Statistical Analysis

The ADFI was calculated as \[(\text{total feed added} - \text{feed weighed back})/(\Sigma p_i d_i)\], where \(p_i\) and \(d_i\) are individual pigs and number of days in the pen, respectively. Mucosal-attached lactic acid bacteria and \(E.~coli\) populations were expressed as colony-forming units per gram of intestinal mucosa. Data were analyzed as a completely randomized design using the MIXED procedures (SAS Inst. Inc.). Pen was considered the experimental unit for all response criteria measured. When a significant \(F\)-value \((P < 0.05)\) for treatment means was observed in ANOVA, treatments were compared using Table 2.

### Performance and Fecal Score of Early Weaned Pigs Fed Different Experimental Diets

| Item                  | PC   | NC   | 7% RPS | 14% RPS | SEM² | \(P\)-value |
|-----------------------|------|------|--------|---------|------|-------------|
| Initial BW, kg        | 6.4  | 6.5  | 6.5    | 6.5     | 0.05 | 0.310       |
| Final BW, kg          | 12.6b| 11.7a| 11.5a  | 11.4a   | 0.15 | <0.001      |
| ADG \(g\) d 0 to 7    | 195a | 140b | 137b   | 127b    | 11.6 | 0.001       |
| d 7 to 14             | 377a | 324a | 330a   | 289b    | 18.6 | 0.024       |
| d 14 to 21            | 295  | 288  | 243    | 296     | 15.8 | 0.075       |
| d 0 to 21             | 289a | 251b | 236b   | 277b    | 5.6  | <0.001      |
| ADG \(g\) d 0 to 7    | 299  | 305  | 278    | 290     | 10.9 | 0.349       |
| d 7 to 14             | 573  | 509  | 492    | 533     | 41.0 | 0.544       |
| d 14 to 21            | 499b | 486a | 414b   | 515a    | 15.5 | <0.002      |
| d 0 to 21             | 457a | 433b | 395b   | 446b    | 16.1 | 0.050       |
| G:F \(g/g\) d 0 to 7  | 0.66a| 0.45b| 0.50b  | 0.44b   | 0.040| 0.001       |
| d 7 to 14             | 0.68 | 0.65 | 0.73   | 0.56    | 0.069| 0.394       |
| d 14 to 21            | 0.59 | 0.60 | 0.59   | 0.58    | 0.039| 0.984       |
| d 0 to 21             | 0.64a| 0.58ab|0.61b  |0.53b   | 0.026| 0.049       |
| Fecal score \(1\)     | 0.19b| 0.76a| 0.09b  | 0.43b   | 0.14 | 0.010       |
| d 7 to 14             | 0.38 | 0.55 | 0.52   | 0.67    | 0.11 | 0.367       |
| d 14 to 21            | 0.40 | 0.60 | 0.37   | 0.71    | 0.14 | 0.296       |
| d 0 to 21             | 0.34a| 0.61a| 0.38b  | 0.63a   | 0.09 | 0.034       |

\(a,b\) Means within rows without common letters differ \((P < 0.05)\).

1Diets: PC = Positive control, diet containing antibiotic; NC = negative control with no potato starch or antibiotic; 7% RPS = negative control + 7% potato starch; 14% RPS = negative control + 14% potato starch.

2\(n = 7\)/group.

3Fecal score: 0, normal; 1, mild diarrhea; 2, moderate diarrhea; 3, severe diarrhea.
Tukey’s test. A LSD multiple comparison test was used to calculate the statistical significance ($P < 0.05$) for the phylogenetic lineage done in molecular analysis.

## RESULTS

### Diet Formulation and Nutrient Analysis

The calculated and analyzed nutrient composition of the experimental diets is shown in Table 1. Diets did not differ in their chemical composition. The analyzed nutrient composition of all diets were greater than the calculated nutrient composition but met the NRC nutrient requirements (NRC, 1998; Table 1).

### Piglet Performance and Fecal Score

The initial BW of the piglets did not differ among dietary treatments (Table 2). Average daily gain was greatest for the PC diet ($P < 0.01$), and the final BW of the PC-fed pigs was greater ($P < 0.01$) than pigs fed the other diets (Table 2). Average daily feed intake did not differ during the first 2 wk of the study, but in the third week pigs fed the 7% diet consumed less feed ($P < 0.01$) compared with the other dietary treatments (Table 2). Overall, the PC and 14% RPS fed pigs consumed more feed ($P < 0.05$) than pigs fed the 7% RPS diet (Table 2). Overall, the G:F was greatest ($P < 0.05$) for the pigs fed the PC and 7% RPS diets, and least with the 14% RPS diet (Table 2).

### Organ Weights

Dietary treatments had no effect on empty or full weights of the gastric stomach, duodenum, jejunum, ileum, or the liver (data not shown).

### Digesta pH, Ammonia N, VFA, Lactic Acid, and Starch Concentration

Dietary treatments had no effect on ileal digesta VFA concentrations, but the ileal lactic acid concentration was greater ($P < 0.05$) for the 14% RPS diet. The colon VFA concentrations were not different across treatments except for valeric acid, which was greatest ($P < 0.05$) for the 14% RPS diet (Table 3). The ileum and colon VFA concentrations are shown in Table 3.
colon digesta pH were not affected by dietary treatments (Table 3). Diets containing RPS had greater \((P < 0.05)\) ileum digesta ammonia N concentrations than diets without RPS; however, the dietary treatments had no effect on the colon digesta ammonia N concentrations (Table 3). Starch content of the ileum was least \((P < 0.05)\) for the PC fed pigs, but was almost 4-fold greater \((P < 0.05)\) for the 14% RPS fed pigs. In contrast, the starch content of the colon was least \((P < 0.05)\) for the 14% RPS fed pigs (Table 3).

Table 4. *Escherichia coli* and lactic acid bacterial (LAB) counts from colon mucosa\(^1\) of early weaned pigs fed different experimental diets\(^2\)

| Item, log cfu g tissue\(^{-1}\) | PC  | NC  | 7% RPS | 14% RPS | SEM\(^3\) | P-value |
|--------------------------------|-----|-----|--------|---------|-----------|---------|
| *E. coli*                      | 6.3\(^b\) | 7.96\(^a\) | 6.93\(^b\) | 7.04\(^b\) | 0.392 | 0.052 |
| LAB                            | 6.55 | 7.26 | 6.98 | 7.24  | 0.360 | 0.481 |
| LAB:*E. coli*                  | 1.05 | 0.91 | 1.03 | 1.05 | 0.058 | 0.283 |

\(^{a,b}\)Means within rows without common letters differ \((P < 0.05)\).

\(^{1}\)The colonic tissue samples taken for microbial analysis were weighed (10 g), washed vigorously with sterile physiological saline to remove nonattached bacteria, and a blunt knife was used to scrape off the epithelial tissue, which was weighed and subsequently diluted 10-fold with PBS, homogenized, and decimally diluted.

\(^{2}\)Diets: PC = Positive control diet containing antibiotic; NC = negative control with no potato starch or antibiotic; 7% RPS = negative control + 7% potato starch; 14% RPS = negative control + 14% potato starch.

\(^{3}\)n = 7/group.

Table 5. Terminal-RFLP-based hierarchical microbial composition of ileum digesta of early weaned pigs fed different experimental diets\(^1\)

| Microbial level,\(^2\) % | PC  | NC  | 7% RPS | 14% RPS | SEM\(^3\) |
|-------------------------|-----|-----|--------|---------|-----------|
| Phylum Bacteroidetes    | 4.0 | 2.2 | 2.8    | 1.5     | 0.5       |
| Class unclassified Bacteroidetes | 4.0 | 2.2 | 2.8    | 1.5     | 0.5       |
| Phylum Firmicutes       | 82.8 | 87.8 | 87.9  | 89.6    | 1.5       |
| Class Bacilli           | 9.1 | 8.8 | 9.2    | 9.0     | 0.1       |
| Order Lactobacillales   | 8.1 | 8.2 | 8.5    | 8.2     | 0.1       |
| Class Clostridia        | 73.7 | 75.8 | 76.6  | 79.9    | 1.3       |
| Order Clostridales      | 69.7\(^b\) | 53.8\(^b\) | 51.5\(^b\) | 78.4\(^a\) | 6.4       |
| Order unclassified Clostridiales | 4.0\(^b\) | 21.4\(^b\) | 25.5\(^b\) | 1.5\(^b\) | 6.1       |
| Class unclassified Firmicutes | 0.0 | 1.6 | 2.1    | 0.7     | 0.5       |
| Class Mollicutes        | 0.0 | 0.5 | 0.0    | 0.0     | 0.1       |
| Order Incertaes Sedis 8 | 0.0 | 0.5 | 0.0    | 0.0     | 0.1       |
| Phylum Actinobacteria   | 1.0 | 0.0 | 0.7    | 0.0     | 0.3       |
| Class Actinobateria     | 1.0 | 0.0 | 0.7    | 0.0     | 0.3       |
| Subclass Actinobacteria | 1.0 | 0.0 | 0.7    | 0.0     | 0.3       |
| Order Actinomycetales   | 1.0 | 0.0 | 0.7    | 0.0     | 0.3       |
| Phylum Proteobacteria   | 11.0 | 10.0 | 7.7    | 8.4     | 0.8       |
| Class Epsilonproteobacteria | 2.0 | 1.1 | 1.4    | 1.5     | 0.2       |
| Order Campylobacterales | 2.0 | 1.1 | 1.4    | 0.0     | 0.4       |
| Class Delta proteobacteria | 2.0 | 1.1 | 1.4    | 0.0     | 0.4       |
| Order Desulfovibionales | 1.0 | 0.5 | 0.7    | 0.0     | 0.2       |
| Class Gammamproteobacteria | 2.0 | 4.9 | 1.4    | 3.7     | 0.8       |
| Order Pasteurellales    | 0.0 | 2.7 | 0.0    | 0.7     | 0.6       |
| Order Enterobacteriales | 2.0 | 1.1 | 0.0    | 3.0     | 0.6       |
| Order Aeromonadales     | 0.0 | 1.1 | 1.4    | 0.0     | 0.4       |
| Class Betaproteobacteria | 3.0 | 1.1 | 2.1    | 1.5     | 0.4       |
| Order Burkholderiales   | 2.0 | 0.5 | 1.4    | 0.7     | 0.3       |
| Order unclassified Betaproteobacteria | 1.0 | 0.5 | 0.7    | 0.7     | 0.1       |
| Class Alphaproteobacteria | 2.0 | 0.5 | 0.7    | 0.7     | 0.3       |
| Order Rickettsiases     | 1.0 | 0.5 | 0.7    | 0.7     | 0.1       |
| Order unclassified Alphaproteobacteria | 1.0 | 0.0 | 0.0    | 0.0     | 0.3       |
| Class unclassified Proteobacteria | 0.0 | 0.5 | 0.7    | 0.7     | 0.2       |
| Phylum Lentisphaerae    | 1.0 | 0.5 | 0.7    | 0.7     | 0.1       |
| Class Lentisphaerae     | 1.0 | 0.5 | 0.7    | 0.7     | 0.1       |
| Order Victivallales     | 1.0 | 0.5 | 0.7    | 0.7     | 0.1       |

\(^{a,b}\)Means within rows without common letters differ \((P < 0.05)\).

\(^{1}\)Diets: PC = Positive control diet containing antibiotic; NC = negative control with no potato starch or antibiotic; 7% RPS = negative control + 7% potato starch; 14% RPS = negative control + 14% potato starch.

\(^{2}\)Proportional microbial distributions.

\(^{3}\)n = 7/group.
Mucosal-Associated E. coli and Lactic Acid Bacteria on Colonic Mucosa

The colonic mucosal microbial data indicated that dietary treatment had no effect \((P < 0.05)\) on the lactic acid bacterial counts or the ratio of lactic acid bacteria to \(E.\ coli\) (Table 4). However, the NC diet resulted in greater \((P < 0.05)\) numbers of colonic \(E.\ coli\) compared with PC diet.

Molecular Microbial Analysis

Changes in microbial composition were less pronounced in the ileum (Table 5) than colon (Table 6). In the ileum there were changes \((P < 0.05)\) in the orders Clostridiales and unclassified Clostridiales (Table 5). In contrast, in the colon there were also changes \((P < 0.05)\) in microbiota composition in the phyla Bacteroidetes, Firmicutes, and Proteobacteria (Table 6). Richness and diversity difference in the colon was reflective of the changes in the phyla Bacteroidetes, Firmicutes, and Proteobacteria, but not in the ileum (Table 7).

DISCUSSION

The purpose of this experiment was to determine whether the addition of RPS to weanling piglet diets without the inclusion of antibiotics would reduce the effects of postweaning diarrhea. Our results indicate that RPS alone does not replace the growth-enhancing effects of subtherapeutic antibiotics, but has some value in reducing scours in the absence of feed medication. The antibiotic containing diet (PC) provided the best piglet growth, but based on the growth efficiency data, inclusion of 7% RPS without the addition of feed antibiotics provides performance comparable with that of the PC.

Kerr et al. (1998) demonstrated that nursery pigs fed 7 or 14% RPS (medicated diets) did not exhibit improved performance compared with the controls at

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### Table 6. Terminal-RFLP-based hierarchical microbial composition of colon digesta of early weaned pigs fed different experimental diets

| Microbial level, \% | PC      | NC      | 7% RPS   | 14% RPS  | SEM^3 |
|---------------------|---------|---------|----------|----------|-------|
| Phylum Bacteroidetes| 2.7abc  | 1.8a    | 5.3bc    | 8.7b     | 1.6   |
| Class unclassified Bacteroidetes | 2.7abc  | 1.8a    | 5.3bc    | 8.7b     | 1.6   |
| Phylum Firmicutes    | 91.8^a  | 90.2^a  | 88.0^a   | 73.9^b   | 4.1   |
| Class Bacilli        | 7.5^c   | 5.5^c   | 18.7^c   | 19.6^c   | 3.7   |
| Order Lactobacillales| 7.5^c   | 5.5^c   | 18.7^c   | 19.6^c   | 3.7   |
| Class Clostridiales  | 82.9^a  | 82.9^a  | 69.3^c   | 54.3^c   | 6.8   |
| Order Clostridiales  | 69.9^a  | 62.7^a  | 64.0^a   | 43.5^b   | 5.7   |
| Order unclassified Clostridiales | 13.0^a  | 20.3^b  | 5.3^c    | 8.7^m    | 3.2   |
| Class unclassified Firmicutes | 1.4^a   | 1.8     | 0.0      | 0.0      | 0.5   |
| Phylum Actinobacteria| 0.0     | 0.9     | 0.0      | 0.0      | 0.2   |
| Class Actinobacteria | 0.0     | 0.9     | 0.0      | 0.0      | 0.2   |
| Subclass Coriobacteridiae | 0.0     | 0.9     | 0.0      | 0.0      | 0.2   |
| Order Coriobacteriales | 0.0     | 0.9     | 0.0      | 0.0      | 0.2   |
| Phylum Proteobacteria | 4.9^a  | 6.0^a   | 5.3^a    | 15.2^b   | 2.5   |
| Class Epsilonproteobacteria | 1.4^ab  | 0.9^ab  | 0.0^c    | 4.3^b    | 0.9   |
| Order Campylobacteriales | 1.4^ab  | 0.9^ab  | 0.0^c    | 4.3^b    | 0.9   |
| Class Deltaproteobacteria | 1.4     | 0.9     | 2.7      | 4.3      | 0.8   |
| Order Desulfovibrionales | 0.7     | 0.5     | 1.3      | 2.2      | 0.4   |
| Class Gammaproteobacteria | 0.0     | 2.3     | 0.0      | 2.2      | 0.7   |
| Order Pasteurellales | 0.0     | 0.5     | 0.0      | 0.0      | 0.1   |
| Order Enterobacteriales | 0.0     | 1.8     | 0.0      | 2.2      | 0.6   |
| Class Betaproteobacteria | 1.4     | 1.4     | 1.3      | 0.0      | 0.2   |
| Order Burkholderiales | 0.7     | 0.9     | 0.0      | 0.0      | 0.2   |
| Order unclassified Betaproteobacteria | 0.7     | 0.5     | 1.3      | 0.0      | 0.4   |
| Class Alphaproteobacteria | 0.0     | 0.0     | 0.0      | 2.2      | 0.6   |
| Order unclassified Alphaproteobacteria | 0.0     | 0.0     | 0.0      | 2.2      | 0.6   |
| Class unclassified Proteobacteria | 0.7     | 0.5     | 1.3      | 2.2      | 0.4   |
| Phylum Lentisphaerae | 0.7     | 0.5     | 1.3      | 2.2      | 0.4   |
| Class Lentisphaerae | 0.7     | 0.5     | 1.3      | 2.2      | 0.4   |
| Order Victivallales | 0.7     | 0.5     | 1.3      | 2.2      | 0.4   |
| Phylum Spirochaetes | 0.0     | 0.5     | 0.0      | 0.0      | 0.1   |
| Class Spirochaetes | 0.0     | 0.5     | 0.0      | 0.0      | 0.1   |
| Order Spirochaetales | 0.0     | 0.5     | 0.0      | 0.0      | 0.1   |

^a–cMeans within rows without common letters differ \((P < 0.05)\).

1Diet: PC = Positive control diet containing antibiotic; NC = negative control with no potato starch or antibiotic; 7% RPS = negative control + 7% potato starch; 14% RPS = negative control + 14% potato starch.

2Proportional microbial distributions.

3n = 7/group.
Table 7. Effect on richness and diversity indices of ileum and colon digesta in early weaned pigs fed different experimental diets

| Item               | PC     | NC     | 7% RPS | 14% RPS | SEM² | P-value |
|--------------------|--------|--------|--------|---------|------|---------|
| Ileum              |        |        |        |         |      |         |
| Richness³          |        |        |        |         |      |         |
| ICE                | 319.8  | 381.4  | 396.2  | 409.5   | 48.2 | 0.231   |
| Chao 2             | 237.3  | 321.0  | 329.4  | 363.4   | 47.0 | 0.078   |
| MMMean             | 248.8  | 321.2  | 291.3  | 363.3   | 73.0 | 0.456   |
| Diversity⁴         |        |        |        |         |      |         |
| Shannon            | 4.6    | 4.4    | 4.2    | 4.2     | 0.3  | 0.510   |
| Simpson            | 231.0  | 269.0  | 247.7  | 298.1   | 32.2 | 0.272   |
| Colon              |        |        |        |         |      |         |
| Richness³          |        |        |        |         |      |         |
| ICE                | 348.2  | 301.2  | 283.0  | 227.5   | 52.1 | 0.124   |
| Chao 2             | 228.1* | 242.2* | 222.8* | 155.9b  | 17.2 | <0.001  |
| MMMean             | 345.4a | 314.4b | 352.0a | 175.5c  | 6.9  | <0.001  |
| Diversity⁴         |        |        |        |         |      |         |
| Shannon            | 4.1ab  | 4.7a   | 3.8b   | 3.7b    | 0.3  | 0.026   |
| Simpson            | 281.3a | 275.1a | 283.5a | 118.5b  | 41.3 | 0.009   |

| a,bMeans within rows without common letters differ (P < 0.05). |
| ¹Diets: PC = Positive control diet containing antibiotic; NC = negative control with no potato starch or antibiotic; 7% RPS = negative control + 7% potato starch; 14% RPS = negative control + 14% potato starch. |
| ²n = 7/group. |
| ³Richness provides a relative index of the number of different species (or taxa) in a sample. Richness estimators are based on the probability that 2 randomly chosen taxa from 2 different samples are the same. The less the probability, the greater the number of different taxa in that sample. The difference between Chao 2, the incidence coverage estimator (ICE), and the Michaelis-Menten Mean (MMMean) is the sampling distribution that is assumed. |
| ⁴A diversity index is richness multiplied by an estimator of abundance and is the probability that 2 taxa drawn from the same sample are the same. The Simpson index is a simple index based on richness and abundance, and Shannon’s index uses a natural logarithmic transformation, which makes it more sensitive to the addition or loss of rare taxa. For example, consider 2 samples have 100 species each. Sample A has 10 species of 10 individuals each. Sample B has 10 species; 9 species have 1 individual each, and 1 species has 91 individuals. Sample A and B have the same richness, but sample A is more diverse. |

35 d postweaning. They also observed an increase in feed intake at the greater RPS concentration, but this did not translate into an improvement in gain or efficiency. Scouring was not measured in their study (Kerr et al., 1998). Callesen et al. (2007) demonstrated that pigs fed diets containing 7.39% potato starch without the inclusion of antibiotics tended to result in better performance and reduced scouring. However, the potato starch pigs also received more treatments of therapeutica. We demonstrated that the inclusion of RPS at 7% reduced scouring, but at 14% the effects were negative.

The negative effects of RPS can potentially be related to the observation that when 14% RPS was included in the diet, there was a large amount of undigested starch in the ileum, but little undigested starch was present in the colon in comparison with the other treatments. The reasons for the high starch content in the ileum, but low concentrations in the colon are unclear, but suggest that the greater concentration of dietary starch is impairing digestion. In contrast, when 7% RPS was included the amounts of starch in the ileum were greater than the PC but in the colon were less than the PC. The differences in gut starch content are not likely the result of fermentation by gut microbiota because total VFA production did not differ among treatments in the ileum or colon.

In a recent experiment (Rideout et al., 2008), 30-kg of BW pigs were fed diets formulated with granular potato starch and compared with diets formulated with conventional ingredients. The potato starch-containing diet resulted in greater (P < 0.05) concentrations of starch in the ileum, but not in the feces. Potato starch decreased (P < 0.05) total apparent tract CP digestibility, and there were no differences in total VFA measured in the cecum, but butyrate did increase. Mentschel and Claus (2003) also demonstrated an increase in butyric acid in pigs fed raw potato starch. However, we showed no changes in butyric acid concentration; however, this may be a function of the age of the pigs.

Culture-independent analysis of 16S rDNA from ileal and colonic contents with T-RFLP demonstrated that the only significant differences in the ileum occurred with the clostridia, but in the colon significant differences were far more widespread. Using culture-based methods, Kleessen et al. (1997) fed rats a diet consisting of 10% raw or retrograded potato starch from 8 d until 5 mo of age and enumerated populations of bacteria. They observed decreased (P < 0.05) fecal numbers of Bacteroides, Lactobacillus, Streptococcus, and Enterococcus in the raw as compared with the retrograded potato starch, but these effects were not observed until 5 mo of age and may have been associated with changes of microbiota associated with age. Bird et al. (2007)
observed greater numbers of lactobacilli, and bifidobacteria in the colon of pigs fed high-amylose resistant starch. A similar result was observed for feces. Wang et al. (2002) also observed increases in lactobacilli and bifidobacteria in the colon.

We observed increased lactobacilli prevalence in the colon when RPS was included in the diet, but because we were using culture-independent methods a much wider range of bacteria could be assessed. Increased numbers of lactic acid bacteria were not observed in our study with culture-based methods. In the colon there was a significantly greater prevalence of lactobacilli in the RPS diets, but not in the ileum. We also observed increased prevalence of Bacteroides, which correlates with the increased numbers of Bacteroides in the colon observed by Kleessen et al. (1997). Kleessen et al. (1997) also observed high numbers of Streptococcus, and Enterococcus that would have fallen within the larger bacterial taxonomic order of bacilli (Cole et al., 2005) as described in our study.

The occurrence of scours in the 14% RPS diet correlated strongly with the decline in richness and diversity of microbial species in the colon. In our own studies (Sepelri et al., 2007; Bhandari et al., 2008) a decline in microbial species richness and diversity was associated with digestive abnormalities. Evidence is accumulating that in many situations a reduction in ecosystem diversity is associated with instability (Kassen and Rainey, 2004; Lozupone and Knight, 2007; Mes, 2008). In microbial ecosystems theory still lags behind that of macro ecosystems, and diversity studies in the pig gut may be an important new tool to predict and understand how diets affect gut health.

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