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Short communication

Characterization of ileal bacterial microbiota in newly-weaned pigs in response to feeding lincomycin, organic acids or herbal extract

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Abstract

The changes of ileal bacterial microbiota in pigs during the first 2 weeks post-weaning in response to feeding lincomycin, organic acids or herbal extract were characterized using 16S rRNA gene-based PCR-denaturing gradient gel electrophoresis (DGGE) profiling, DNA sequencing, and real-time PCR (QPCR) techniques. Both time post-weaning and the dietary treatments resulted in a shift of the microbiota composition. While time post-weaning mostly influenced *Clostridium* group, the feed additives increased the population of *Lactobacillus* and related lactic acid bacteria by ≥3-fold.

Keywords: Pigs; Ileal microbiota; Lincomycin; Organic acids; Herbal extract; 16S rRNA

1. Introduction

Piglets are under stress at weaning. The stressors include changes in diet composition, environment and bacterial challenges and contribute to digestive upsets and depressed growth rates (Pluske et al., 2003). Dietary antibiotics have been used to largely overcome weaning-associated disorders, such as diarrhea caused by *Escherichia coli* K88 (Bosi et al., 2004), which significantly affect animal productivity. However, this practice has been forbidden today in Europe due to public concerns over the potential link of the use of antibiotics in feed to the wide spread of bacterial antibiotic-resistance that threatens human health. To develop viable alternatives to dietary antibiotics, various feed additives such as organic acids, copper sulphate, zinc oxide, probiotics, prebiotics, and herbs have been tested on newly-weaned piglets (NRC, 1998). Partanen and Mroz (1999) and Piva et al. (2002) reported that the inclusion of organic acids in the diet can enhance growth performance and modulate swine intestinal fermentation and microbial proteolysis. Lactic acid in particular has been reported to reduce proliferation of an enterotoxigenic *E. coli* (Thomlinson and Lawrence, 1981) and to be more effective than other organic acids in improving pig growth performance (Tsiloyiannis et al., 2001). Recently we also observed that supplementing diets with lincomycin and a blend of acids, containing a

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large amount of lactic acid, showed a similar degree of growth promotion in pigs during week 2 post-weaning. This observation coincided with changes in the ileal bacterial microbiota that were not investigated in detail (Namkung et al., 2004). The objective of the present study was to characterize the changes of ileal microbiota in response to the dietary treatments, including analyses of the diversity of the microbiota and identification of affected bacteria.

2. Materials and methods

2.1. Animals, diets, and sample collection

Ileal digesta samples were obtained from pigs that were used in a large-scale pig performance study. General experimental procedures, including a detailed description of dietary treatments, and growth performance data have been reported previously (Namkung et al., 2004). Briefly, pigs were not fed creep feed before weaning, weaned at 16 to 19 days of age with an average body weight (BW) of 4.90±0.67 kg. Pigs were exposed to one of five dietary treatments, with two pens of six pigs per treatment in each of three equal blocks: 1) basal diet (Pig Starter); 2) basal diet with lincomycin (110 mg/kg); 3) basal diet with a herbal extract (0.75%); 4) basal diet with acid blend 1 (1.1%; Acids-1); and 5) basal diet with acid blend 2 (2.1%; Acids-2). Treatments 1 and 2 served as a negative and a positive control, respectively. At day 14 post-weaning, one pig from each of two pens per treatment and with growth rate closest to the pen average was sacrificed for sampling of ileal digesta. Digesta samples from two pigs of the same treatment were pooled. In addition, ileal digesta was obtained and pooled from five pigs just before weaning. Collection and preparation of the digesta samples from the pig ileum for bacterial DNA extraction were conducted as described previously (Li et al., 2003).

2.2. Cell lysis, DNA extraction, and PCR–DGGE analysis

Cell lysis, chromosomal and plasmid DNA extraction, PCR amplification, DGGE electrophoresis of PCR amplicons, and visualization of DNA bands were conducted as described previously (Li et al., 2003). PCR primers were HDA1-GC (5′-**CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG G**AC TCC TAC GGG AGG CAG CAG T-3′; the GC clamp is in boldface) and HDA2 (5′-GTA TTA CCG CGG CTG CTG GCA C-3′) (Walter et al. (2000) against the V3 region of the 16S rRNA genes (position 339 to 539 in the *E. coli* gene) of bacteria.

The similarities of PCR–DGGE profiles were analyzed with BioNumerics software version 3.0 (Applied Maths, Sint-Martens-Latem, Belgium) using the Dice function. DNA bands were manually assigned in the software and compared using a positional tolerance of 0.5% with manual correction where required. A distance matrix was calculated by the Dice and dendrograms were constructed from this matrix using the unweighted pair group mean average (UPGMA). The degree of similarity was represented by a similarity coefficient. Both Shannon Index and Simpson's Diversity Index were determined based on the presence and density of DNA bands using the R vegan Package (Version 1.8-8) (Oksanen et al., 2007).

2.3. Isolation and sequence analysis of DNA bands from DGGE gels

To determine the bacteria represented by DNA bands in DGGE gels that were either major representative bands or

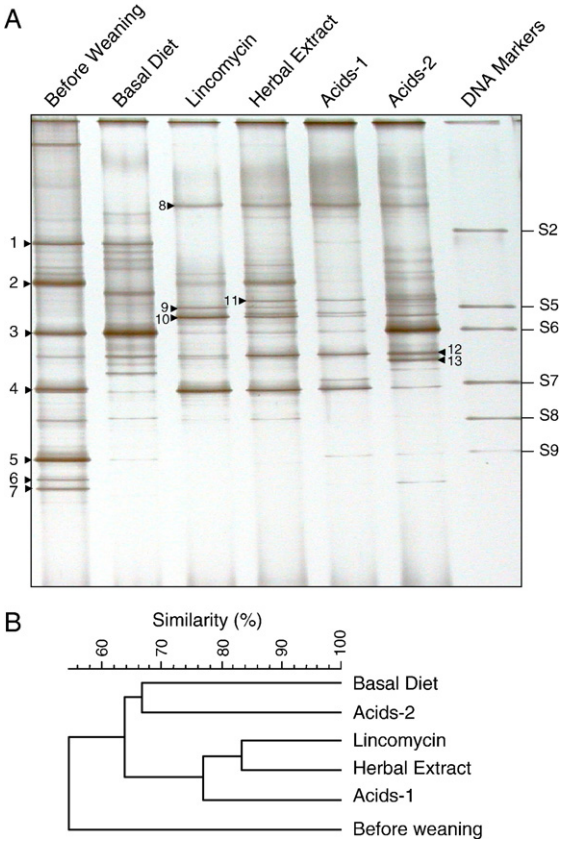

Fig. 1. Effect of weaning, dietary lincomycin, and feeding blends of herbal extracts and organic acids on the composition of ileal bacterial microbiota. The pigs at 2-weeks post-weaning were fed the diets of Pig Starter containing no antibiotics (Basal diet), lincomycin at 110 mg/kg, or feed blends of herbal extracts or organic acids (Acids-1 and Acids-2). (A) PCR–DGGE bacterial profiles of ileal bacterial microbiota. DNA bands #1–13: The DNA bands have been recovered from the gel and sequenced (see Table 1). DNA Markers: partial 16S rRNA gene amplicons from the cultures of selected bacteria; S1, *Lactobacillus amylovorus*; S2, *Bacillus subtilis*; S3, *E. coli* O157:H7; S4, *Clostridium perfringens*; S5, *Salmonella typhimurium*; S6, *Clostridium lituseburense*. Note: A similar image of PCR–DGGE profiles has been published previously. The use of the image in this report is only for a demonstration of DGGE DNA bands. (B) UPGMA tree representing the relatedness of PCR–DGGE profiles of ileal bacterial microbiota. The tree was generated based on a distance matrix calculated by the Dice.