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Evaluation of probiotic bacteria for their effects on the growth performance and intestinal microbiota of newly-weaned pigs fed fermented high-moisture maize

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ABSTRACT

The effects of feeding high-moisture maize fermented with *Lactobacillus acidophilus* or *Pediococcus acidilactici* on the growth performance and intestinal microflora of newly weaned pigs were investigated. A total of 270 newly weaned pigs (equal numbers of castrated males and females) were allocated to 3 dietary treatments (5 pens per treatment and 18 pigs per pen) in a randomized complete block design on the basis of body weight (BW) and litter. The dietary treatments included a basal diet supplemented with: 1) naturally fermented high-moisture maize without any microbial inoculants (Control); 2) high-moisture maize fermented with *L. acidophilus* (LA); and 3) high-moisture maize fermented with *P. acidilactici* (PA). Pigs were fed diets in liquid form for a 5-wk period. Quantitative polymerase chain reaction (QPCR) analysis with PCR primers specific to *L. acidophilus* or *P. acidilactici* indicated a well-developed bacterial population of each inoculated bacterium in the fermented high-moisture maize. Lactic acid was the major short-chain fatty acid (SCFA) produced from the fermentation. Among the 3 treatments and on most sampling days, LA-fermented maize had the numerically highest concentration of lactic acid. Dietary treatments over the 5-wk period did not impact pig growth performance. However, during last 3 wks of the trial, pigs on the LA treatment had higher BW gains than pigs on PA. Pigs fed LA-fermented high-moisture maize showed fewer coliform bacteria in fecal samples when compared to pigs fed PA-fermented high-moisture maize ($P<0.05$). The PCR and denaturing gradient gel electrophoresis (DGGE) analysis on the relatedness of bacterial profiles of colonic microbiota revealed 3 bacterial clusters and the microbiota from PA-treated pigs formed 1 cluster. In addition, feeding LA- or PA-fermented maize reduced the diversity and richness of bacterial microbiota in the colon ($P<0.05$). Both *L. acidophilus* and *P. acidilactici* colonized poorly in the intestine of pigs regardless of the dietary treatments. These results indicate that feeding the probiotic-fermented, high-moisture maize can modulate the intestinal microbiota, and LA has the potential to impact pig growth performance.

1. Introduction

Early weaning is an effective way to enhance breeding efficiency and economical profits in modern intensive swine production (King et al., 1999; Yao et al., 2008). However, early weaning can induce stress that not only impairs intestinal function but also compromises immunity of pigs (Pié et al.,

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2004; Tang et al., 2005; Wu, 1998). Weaning is thus associated with growth retardation and increases in both morbidity and mortality in piglets (Wilson et al., 1989). It is generally accepted that the enhancement of intestinal health of weanling pig is critical to prevent or attenuate the occurrence of disease resulted from the continual exposure of weanling piglets to a wide variety of pathogenic microorganisms (Han et al., 2007). Probiotics can enhance intestinal health by stimulating the development of a healthy microbiota (predominated by beneficial bacteria), preventing enteric pathogens from colonizing the intestine, increasing digestive capacity and lowering the pH, or improving mucosal immunity (de Lange et al., 2010). In the past, lactic acid bacteria (LAB) and *Bacillus* species have largely been used as probiotic bacteria for food animal production. *Lactobacillus* and *Pediococcus* belong to the group of LAB. They have a role in maintaining a healthy intestinal microbial ecosystem (Alande et al., 1999; Walter, 2008; Lessard et al., 2009; Molly et al., 1996).

Recently, probiotic products containing strains of *L. acidophilus* (Chris Hansen A/S, Hørsholm Denmark) or *P. acidilactici* (Lallemond S.A.S., Blagnac Cedex, France) have been developed for the use in swine production. In the province of Ontario, Canada, ensiled high-moisture maize is a major energy source in commercial pig diets, and the feeding value of high-moisture maize may be enhanced through the use of exogenous enzymes or microbial inoculants in liquid feeding systems (Niven et al., 2007). Liquid feeding offers a number of advantages to swine production. The practice can facilitate the use of liquid co-products and high-moisture grains in pig diets. It also allows the use of exogenous enzymes or microbial inoculates to manipulate the feeding value of feed ingredients (Brooks et al., 2001). In the present study, we used a liquid feeding system and evaluated 2 commercially available probiotic products for their potential in improving the growth performance and intestinal health of newly weaned pigs through feeding fermented high-moisture maize.

2. Materials and methods

2.1. Bacteria and growth

The probiotic products containing *L. acidophilus* [2×10^{10} colony-forming unit (CFU)/g] or *P. acidilactici* (Bactocell, 1×10^{10} CFU/g) were obtained from commercial companies (Chris Hansen A/S, Hørsholm, Denmark and Lallemand S.A.S., Blagnac Cedex, France, respectively). The dry products were added to the mixture of high-moisture maize and water for fermentation experiments. In the laboratory, pure cultures of the bacteria were grown in the MRS medium at 37 °C in an anaerobic chamber (Coy Laboratory Products, Grass Lake, MI, USA) with 80% N_2, 10% CO_2, and 5% H_2 atmosphere.

2.2. Preparation of fermented maize

Individual batches of 800 kg high-moisture maize that had been stored unground in sealed silos were finely ground and immediately transferred into separate 3000-L fermentation tanks (swine liquid feeding unit, Arkell Swine Research Station, University of Guelph, Guelph, ON, Canada). The maize was mixed with water (maize:water = 1:2) and inoculated with either *L. acidophilus* (LA) or *P. acidilactici* (PA) at the concentration of 1×10^7 CFU/mL or stored without any inoculation (Control). The level of inoculation was based on the report on the production of lactic acid during the fermentation with *L. acidophilus* and *P. acidilactici*, as described previously (Niven et al., 2006, 2007). The fermentation tanks were maintained at a room temperature of approximately 20 °C and high-moisture maize was taken from the appropriate fermentation tanks at each feeding, mixed with the other diet components and delivered immediately to the pigs. The initial batches were prepared 2 d prior to the start of the pig growth performance study. Additional batches of high-moisture maize were prepared on d 29 and 38 of the growth performance study, reflecting feed consumption by the experimental pigs. Samples were collected weekly from the fermentation tanks for analysis of lactic acid content as described by Niven et al. (2004) using ion exclusion chromatography.

2.3. Animal trial and sample collection

The pigs used in this study were purebred Yorkshire and maintained at the Arkell Swine Research Station. The protocol for the animal experiment was approved by the Animal Care and Use Committee of University of Guelph. Pig growth performance was monitored during the first 5-wk post-weaning.

Two hundred and seventy piglets (equal numbers of castrated males and females; 3 equal consecutive weekly blocks), weaned at age of 18 to 21 d with an average initial body weight of 7.29 ± 0.09 kg, were allocated to 3 dietary treatments (5 pens per treatment with 9 males and 9 females per pen) on the basis of weight, sex and litter of origin in a randomized complete block design. The dietary treatments were liquid feeding of a basal diet supplemented with: 1) naturally fermented high-moisture maize without any microbial inoculants (Control); 2) high-moisture maize fermented with *L. acidophilus* (LA); and 3) high-moisture maize fermented with *P. acidilactici* (PA). Feed was offered in six equal meals daily using a computer controlled liquid feeding system (Hydrojet Big Dutchman, Vechta, Germany) and as described in detail by Columbus et al. (2010). Feeding levels were gradually increased and adjusted for individual pens to maximize feed intake, while minimizing feed spillage. Additional water was freely available. The room temperature was maintained between 22 and 25 °C and additional heat was provided from 2 heat lamps in each pen. Each of the 2 rooms had 12 identical pens and a central alleyway. The pens adjacent to the entrance of the room and against the outside walls were not used to minimize climatic variation between pens. Within each room, 2 or 3 pens were assigned to the dietary treatments.

Pigs were fed according to a typical, 3-phase feeding program (Table 1). Dietary crude protein, indispensable amino acids, vitamins, and minerals were supplemented to meet or exceed established nutritional requirements for each phase (NRC, 1998). All diet components, except for fermented high-moisture maize, were pre-mixed and crumbled at the feed mill at the Arkell Swine Research Station. The feed was prepared by mixing the feed components with fermented high-moisture maize in a computer controlled feed mixing tank before transfer to feeding troughs in each pens (Columbus et al., 2010). No antibiotic growth promoters were added to the diets. The diets were gradually transitioned from the first phase to the second phase between 10th and 14th day,