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Bacteriophage and probiotics both enhance the performance of growing pigs but bacteriophage are more effective☆

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ABSTRACT

Two experiments were conducted to determine the effects of dietary supplementation with bacteriophage, probiotics and their combination on growth performance, apparent total tract digestibility (ATTD), fecal bacterial populations and serum immunoglobulins in growing pigs. In both experiments, 200 barrows (Landrace×Yorkshire×Duroc) were randomly allotted to 4 treatments on the basis of BW. There were 5 replicate pens in each treatment with 10 pigs per pen. Experimental diets were fed in meal form for 35 d. In Exp. 1, dietary treatments included basal diet supplemented with 0 (control diet without any antimicrobials), 0.5, 1.0 and 1.5 g/kg commercial bacteriophage product. Bacteriophage product contained a cocktail of bacteriophages of Salmonella (*Salmonella typhimurium*, *Salmonella enteritidis*, *Salmonella cholerasuis* and *Salmonella derby*), *Staphylococcus aureus*, *Escherichia coli* and *Clostridium perfringens* types A and C. Dietary increasing levels of bacteriophage linearly improved (P<0.05) the ADG, ADFI and ATTD of DM. At d 35 of the experiment, pigs fed diets supplemented with increasing levels of bacteriophage had greater (linear, P<0.05) fecal TAB, *Bifidobacterium* spp. and *Lactobacillus* spp. and fewer (linear, P<0.05) fecal *Clostridium* spp. and coliforms. Dietary treatments had no effect (P>0.05) on serum immunoglobulin concentrations at d 35 of experiment. In Exp. 2, dietary treatments were basal diet without any antimicrobials (Control) and basal diets supplemented with 3.0 g/kg fermented probiotic product (P), 1.0 g/kg bacteriophage (B) and combination of 1.0 g/kg bacteriophage and 3.0 g/kg fermented probiotic product (BP). Probiotic products used herein contained *Lactobacillus acidophilus*, *Bacillus subtilis* and *Saccharomyces cerevisiae*. Pigs fed the B and BP diets had greater (P<0.05) ADG, ADFI, G:F and ATTD of DM, CP and GE than that of pigs

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain apparent total tract digestibility; BW, body weight; CP, crude protein; DM, dry matter; G:F, gain:feed; GE, gross energy; NC, negative control; PC, positive control; TAB, total anaerobic bacteria.

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fed the control and P diets. Pigs fed the P diet had greater (P<0.05) ADG, ADFI and ATTD of CP than that of pigs the fed control diet. At d 35, pigs fed the BP diet had greater (P<0.05) fecal TAB, *Bifidobacterium* spp. and *Lactobacillus* spp. and fewer (P<0.05) *Clostridium* spp. and coliforms than pigs fed the control diet. Also, pigs fed the P and B diets had greater (P<0.05) *Lactobacillus* spp and fewer (P<0.05) coliforms at d 35 than that of pigs fed the control diet. Dietary treatments had no effect (P>0.05) on serum immunoglobulin concentrations (d 35). The present results suggest that bacteriophages and probiotics both improve different aspects of grower pig's performance but that bacteriophages are more effective than probiotics and would appear to offer an alternative to antibiotic type growth promoters.

1. Introduction

In South Korea, use of antibiotics as growth promoters in animal feeds has been forbidden since 2011 (Global Agricultural Information Network, 2011). Therefore, the search continues for non-antibacterial growth promoters that are active *in vivo*, are fast acting, possess a broad spectrum in activity, do not induce bacterial resistance and subsequently promote growth performance of pigs. A number of research findings on the use of alternatives like probiotics, oligosaccharides, organic acids and antimicrobial peptides to replace antibiotics in feed have been documented with varying success (Choi et al., 2011a; Yan et al., 2011; Yoon et al., 2012, 2013; Lee et al., 2014). In this context, bacteriophages are believed to be an ideal candidate, due to their natural antibacterial properties (Jamalludeen et al., 2009; Yan et al., 2012; Wang et al., 2013).

Bacteriophages are obligate intracellular parasites that multiply inside bacteria by making use of some or all of the host biosynthetic machinery (McGrath et al., 2004). Bacteriophages are amongst the most abundant living entities on earth playing important roles in maintaining the natural abundance and distribution of microorganisms (Sulakvelidze, 2011) and have been used to both prevent and treat bacterial diseases in human and animals. Most of the previous studies on bacteriophages evaluated their therapeutic effects on disease challenged pigs (Barrow, 2001; Jamalludeen et al., 2009; Wall et al., 2010) and poultry (Huff et al., 2002; Toro et al., 2005; Atterbury et al., 2007). Previous studies with dietary supplementation of anti-*Salmonella* bacteriophage reported improved performance and reduced bacterial shedding in growing pigs (Gebru et al., 2010; Yan et al., 2012). Recently it has been reported that supplementation with bacteriophages to laying hens diets resulted in greater feed efficiency, egg production and improved excreta microbiota (Zhao and Kim, 2012; Wang et al., 2013). Previous studies in the authors laboratory reported that a multimicrobe probiotic products had potential to improve the performance and gut health and could be used as an alternative to antibiotics growth promoters in pigs and broilers (Choi et al., 2011b; Kim et al., 2012). The present study was designed to investigate the effects of dietary supplementation with bacteriophage, probiotics and combination of bacteriophage and probiotics on growth performance, ATTD of nutrients, fecal bacterial populations and serum immunoglobulins of growing pigs.

2. Materials and methods

The project underwent proper ethical standards and the experiments were approved by the Institutional Animal Care and Use Committee of Kangwon National University, Chuncheon, Republic of Korea. These experiments were conducted at the facility of Kangwon National University farm and the pigs (Landrace×Yorkshire×Duroc) were housed in partially slotted and concrete floor pens with a pen size of 2.80 m×5.00 m. All pens were equipped with a self-feeder and nipple drinker to allow *ad libitum* access to feed and water.

2.1. Bacteriophage

Bacteriophage product was obtained from a commercial feed company (CTC Bio, Inc., Seoul, Republic of Korea). In short, the product contained a cocktail of bacteriophages of Salmonella (*Salmonella typhimurium*, *Salmonella enteritidis*, *Salmonella cholerasuis* and *Salmonella derby*), *Staphylococcus aureus*, *Escherichia coli* (k88, k99 and f41) and *Clostridium perfringens* types A and C. The product contained 10^9 plaque-forming units (pfu)/g bacteriophages.

2.2. Preparation of probiotic product

Lactobacillus acidophilus isolated from faces of weaned pigs, *Bacillus subtilis* isolated from natto (fermented soybeans), and *Saccharomyces cerevisiae* isolated from koji (malted wheat) were maintained in the laboratory as stock culture. A culture broth (CB) medium containing 60.0 ml corn steep liquor, 40.0 ml molasses, 3.0 g/l yeast extract, 5.0 g/l KH_2PO_4 and 2.5 g/l K_2HPO_4 in distilled water was prepared and autoclaved before being used.

Two liters of autoclaved CB were inoculated with 2.0 mL of culture of each microbe separately and subjected to fermentation for 48 h. *L. acidophilus* and *B. subtilis* were incubated at 37 °C at pH 7.0, whereas *S. cerevisiae* was incubated at 32 °C at pH 4.0. The microbes grown on CB were directly sprayed on corn–soybean meal (1:1) followed by drying at 40 °C for 72 h.