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Effects of dietary supplementation of antimicrobial peptide-A3 on growth performance, nutrient digestibility, intestinal and fecal microflora and intestinal morphology in weanling pigs

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ABSTRACT

In this study, effects of dietary supplementation of the antimicrobial peptide-A3 (AMP-A3) on growth performance, coefficient of total tract apparent digestibility (CTTAD) of nutrients, serum immunoglobulins, intestinal and fecal microflora and intestinal morphology in weanling piglets were evaluated when used as a substitute to the antibiotics. A total of 240 weanling piglets (Landrace × Yorkshire × Duroc, initial body weight (BW): 5.74 ± 0.38 kg) were randomly allotted to 4 treatments on the basis of BW. There were 4 replicate pens in each treatment with 15 pigs per pen. The dietary treatments were: PC (positive control; basal diet + 1.5 g apramycin/kg diet) and AMP-A3 (basal diet supplemented with 0, 60 and 90 mg AMP-A3/kg diets). The experimental diets were fed in a meal form for 2 phases (d 0–14, phase I and d 15–28, phase II post-weaning). Pigs fed the PC diet had greater (P<0.05) overall average daily gain (ADG), average daily feed intake (ADFI) and the CTTAD of crude protein (CP, phase I) and dry matter (DM) and CP (phase II) than pigs fed the AMP-A3 diet. Increasing levels of dietary AMP-A3 linearly improved (P<0.05) overall ADG and CTTAD of DM and CP (phase I and II). Pigs fed the PC diet had lower (P<0.05) coliforms (cecum and feces) and total anaerobic bacteria (TAB, cecum) than pigs fed the AMP-A3 diet. When pigs offered increasing levels of the AMP-A3 diets, there was a linear decline (P<0.05) in TAB, coliforms and *Clostridium* spp. in the ileum, cecum and feces (d 14 and 28). Pigs fed the PC diet had lower (P<0.05) crypt depth and greater (P<0.05) villus height to crypt depth ratio (VH:CD) of the jejunum than pigs fed the AMP-A3 diets. Increasing levels of AMP-A3 in the diets of pigs increased (linear, P<0.05) villus height and VH:CD and decreased (linear, P<0.05) crypt depth of the duodenum and jejunum. Dietary treatments had no effect (P>0.05) on the coefficient of ileal apparent digestibility of amino acids and serum immunoglobulin concentrations. The results obtained in the present study indicate that the AMP-A3 had beneficial effects on growth performance, CTTAD of nutrients, intestinal morphology and intestinal and fecal microflora and can be used as a potential alternative to antibiotic growth promoters in weanling pigs.

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; AMP-A3, antimicrobial peptide-A3; ATTD, 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; VH:CD, villus height:crypt depth ratio.

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1. Introduction

Many changes associated with weaning expose young piglets to a number of stressors that can lead to depressed feed intake, growth performance and increased disease and mortality (Okai et al., 1976; Pluske et al., 1996). This has led to the development of feed additives with high efficiency and low toxicity in order to boost host defense system of weanling pigs. Antibiotics are generally added to the weanling piglet diets to maintain the health and improve growth performance. Nevertheless, their continuous use and misuse has led to the emergence of drug resistance (Monroe and Polk, 2000) and risk of the antibiotic-residues in animal products (Schwarz et al., 2001). Therefore, the search continues for new antimicrobials that are active *in vivo*, are fast acting and broad-spectrum, do not include bacterial resistance and have limited side effects. In this sense, synthetic congeners of the natural antimicrobial peptides (AMP) are believed to be one of the ideal candidates, due to their natural antimicrobial properties, broad spectrum activity, speed of action and a low propensity for the development of bacterial resistance (Hancock and Lehrer, 1998; Bradshaw, 2003). It has speculated that unlike currently used antibiotics, acquisition of resistance by microbes against AMP is thought to be improbable, as AMP have numerous targets and making elimination of one target is less significant (Marr et al., 2006).

The AMP is small gene-encoded peptides that show a broad range of activity against gram-negative and gram-positive bacteria, fungi, and mycobacteria (Zasloff, 2002). The AMP acts against target organism either by membrane depolarisation, micelles formation or diffusion of AMP onto intracellular targets (Matsuzaki, 1999; Shai, 1999; Huang, 2000; Keymanesh et al., 2009). The interest of AMP as potential antibiotic pharmaceuticals has always been high. Because of their rapid and broad spectrum properties, these peptides were quickly proposed as antimicrobials to treat microbial infections, particularly those caused by antibiotic resistant bacteria (Hadley and Hancock, 2010). However, the use of AMP as feed additive is still in the stage of infancy with most of the work being conducted *in vitro* and very few animal studies have been documented. Positive effect of supplementation of various AMP on growth performance (Shan et al., 2007; Wang et al., 2011), nutrient digestibility (Jin et al., 2008a,b), intestinal microflora (Wang et al., 2007; Jin et al., 2009), intestinal morphology (Wang et al., 2006, 2007) and immune functions (Shan et al., 2007; Tang et al., 2009) has been reported previously. The objectives of the present experiment were to evaluate the effects dietary supplementation of synthetic antimicrobial peptide-A3 (AMP-A3) on growth performance, coefficient of total tract apparent digestibility (CTTAD) of nutrients, serum immunoglobulins, intestinal and fecal microflora and intestinal morphology in weanling pigs when it was used as an alternative to antibiotics.

2. Materials and methods

The protocol for the present experiment was approved by the Institutional Animal Care and Use Committee of Kangwon National University, Chuncheon, Republic of Korea. The experiment was conducted at the facility of Kangwon National University farm and the pigs (Landrace × Yorkshire × Duroc) were housed in partially slatted concrete floor pens with pen size 1.90 m × 2.54 m. All pens were equipped with a self-feeder and nipple drinker to allow *ad libitum* access to feed and water.

2.1. Peptide synthesis

The antimicrobial peptide (AMP-A3) used in the present study was provided by Research Center for Proteineous Materials, Chosun University, Kwangju, South Korea. The AMP-A3 (amino acid sequence: AKKVFKRLEKLFSKIWNWK-NH_2) is an analog of antimicrobial peptide HP 2-20 (amino acid sequence: AKKVFKRLEKLFSKIQNDK-NH_2) designed by substitution of amino acid tryptophan for the hydrophobic amino acids, glutamine and aspartic acid (Lee et al., 2002). In short, the AMP-A3 was synthesised by solid phase method using 9-fluorenyl-methoxycarbonyl (Fmoc) chemistry (Merrifield, 1986). Rink amide 4-methyl benzhydrylamine (MBHA) resin (0.55 mmol/g) was used as the support to obtain a C-terminal amidate peptide. The coupling of Fmoc-L-amino acids was performed with N-hydroxybenzotriazole (HOBt) and dicyclohexylcarbodiimide (DCC). Amino acid side chains were protected as follows: tert-butyl (aspartic acid), trityl (glutamine), tert-butyloxycarbonyl (lysine). Deprotection and cleavage from the resin were carried out using a mixture of trifluoroacetic acid, phenol, water, thioanisole, 1,2-ethandithiol and triisopropylsilane (88:2.5:2.5:2.5:2.5:2.0, v/v) for 2 h at room temperature. The crude peptide was then repeatedly washed with diethylether, dried in vacuum, and purified using a reversed phase preparative HPLC on a Waters 15 Am Deltapak C18 column (19 cm × 30 cm). Purity of the peptide was checked by analytical reversed-phase HPLC on an Ultrasphere C18 column (Beckman, Fullerton, CA, USA), 4.6 cm × 25 cm.

2.2. Animals and experimental design

A total of 240 weanling piglets (Landrace × Yorkshire × Duroc; average initial body weight (BW): 5.74 ± 0.38 kg) were randomly allotted to 4 treatments on the basis of BW. There were 4 replicate pens in each treatment with 15 pigs per pen. The dietary treatments were: PC (positive control; basal diet + 1.5 g apramycin/kg diet) and AMP-A3 (basal diet supplemented with 0, 60 and 90 mg AMP-A3/kg diet). The antibiotic used in present study was 0.15% apramycin (Apralan; KBNP Inc., Gunpocity, Kyungki-Do, Korea). The levels of the AMP-A3 used in present study were based upon the results of minimum inhibitory concentration (Lee et al., 2002). The experimental diets were fed in 2 phases: phase I (d 0–14 post-weaning) and phase II (d 15–28 post-weaning). Diets were formulated to contain 14.28 MJ/kg ME and 15.5 g/kg lysine (phase I) and 14.11 MJ/kg