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Effects of dietary supplementation of synthetic antimicrobial peptide-A3 and P5 on growth performance, apparent total tract digestibility of nutrients, fecal and intestinal microflora and intestinal morphology in weanling pigs

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ABSTRACT

The present study investigated the effects of dietary supplementation of synthetic antimicrobial peptide-A3 and P5 (AMP-A3 and P5) on growth performance, apparent total tract digestibility (ATTD) of nutrients, fecal and intestinal microflora, serum immunoglobulins and intestinal morphology of weanling pigs. Pigs ($n=240$; Landrace × Yorkshire × Duroc, initial body weight: 5.9 ± 0.56 kg) were randomly allotted to four treatments on the basis of BW. There were four replicates in each treatment with 15 pigs per replicate. Dietary treatments were negative control (NC; basal diet without antimicrobial growth promoters), positive control (PC; basal diet + 150 mg/kg diet avilamycin), basal diet with 60 mg/kg AMP-A3 (A3) and basal diet with 60 mg/kg AMP-P5 (P5). Diets were fed for 28 d. The overall ADG and G:F of pigs fed PC, A3 and P5 diets were greater ($P<0.05$) than pigs fed the NC diet. Also, the overall ADG of pigs fed the PC diet was greater ($P<0.05$) than pigs fed the A3 and P5 diets. The G:F of the pigs fed the PC diet was greater ($P<0.05$) than pigs fed the A3 diet, whereas, G:F of pigs fed the P5 diet was not different ($P>0.05$) from pigs fed the PC diet. Pigs fed the PC, A3 and P5 diets had greater ($P<0.05$) ATTD of GE, CP (phase I and II) and DM (phase II) than pigs fed the NC diet. Moreover, ATTD of CP (phase I and II) and GE (phase II) of pigs fed the PC diet was greater ($P<0.05$) than pigs fed the A3 and P5 diets. At d 28, pigs fed the PC, A3 and P5 diets had fewer ($P<0.05$) fecal *Clostridium* spp. and coliforms than pigs fed the NC diet. Moreover, pigs fed the PC and P5 diets had fewer ($P<0.05$) ileum and cecal total anaerobic bacteria, *Clostridium* spp. and coliforms than pigs fed the NC diet. Pigs fed the PC, A3 and P5 diets had greater ($P<0.05$) villus height and villus height:crypt depth (VH:CD) of duodenum and jejunum than pigs fed the NC diet. Dietary treatments had no effects ($P>0.05$) on apparent ileal amino acids digestibility and serum immunoglobulins (IgG, IgA, IgM) concentrations. These results indicate that AMP-A3 and P5 have potential to improve the growth performance, nutrient digestibility, intestinal morphology and to reduce pathogenic bacteria in weanling pigs. The impact on several of the measured response parameters was quantitatively low, though. Therefore, further studies are needed to confirm the current results.

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1. Introduction

At weaning, piglets are exposed to many potentially harmful stressors, including separation from the dam, being moved and transition from a liquid to solid diets. These lead to diarrhea (Hampson, 1994; Madec et al., 2000), alteration in structure and function of small intestine (Hampson, 1986; Hampson and Kidder, 1986) and impairment of immune functions (Blecha et al., 1983; Wattrang et al., 1998). To counteract these effects, commercially available antibiotics feed additives are supplemented to weaning pig diets to maintain the health and to improve the growth performance. However, their repeated use/misuse has resulted in development of resistance to antibiotics (Barton, 2000; Hinton et al., 1986). Therefore, there is urgent need for novel, effective and safe antimicrobial growth promoters. In this context, synthetic congeners of the natural antimicrobial peptides (AMPs) are believed to be one of the novel candidates, due to their natural antimicrobial properties, broad spectrum activities, speed of action and a low propensity for the development of bacterial resistance (Bradshaw, 2003; Hancock and Lehrer, 1998).

The AMPs are a vast group of molecules widely distributed throughout nature and produced by prokaryotes and eukaryotes (Maroti et al., 2011). They are small gene-encoded peptides that show a broad range of activity against gram-negative and gram-positive bacteria, fungi, and mycobacterium (Zasloff, 2002). Because of their broad spectrum properties and low propensity for the development of bacterial resistance AMPs were proposed as antimicrobials to treat microbial infections (Hadley and Hancock, 2010). However, high cost of production, toxicity against eukaryotic cells, susceptibility to proteolytic degradation and development of allergies to the peptides are major obstacles in commercial uses of AMPs (Bradshaw, 2003). These problems can be solved by development of synthetic AMPs by alteration in amino acids sequence of natural AMPs and application of recombinant DNA techniques for commercial production of AMPs (Bradshaw, 2003). Recent studies in author's lab have been reported that supplementation of different levels of synthetic AMP-A3 (Yoon et al., 2012) and AMP-P5 (Yoon et al., 2013) to weanling pigs diets had beneficial effects on nutritional performance of pigs. The aim of the present study was to investigate the comparative effects of dietary inclusion of synthetic AMPs (A3 and P5) and antibiotics on growth performance, apparent nutrients digestibility, fecal and intestinal microflora and intestinal morphology of weanling pigs.

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. This 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 3 × 3 m. All pens were equipped with a self-feeder and a nipple drinker to allow ad libitum access to feed and water.

2.1. Peptide synthesis

The antimicrobial peptides (AMP-A3 and P5) used in the present study were provided by Research Center for Protineous Materials, Chosun University, Kwangju, Republic of Korea. In short, the AMP-A3 (AKKVFKRLEKLFSKIWNWK-NH_2) is an analog of antimicrobial peptide HP 2–20 (AKKVFKRLEKLFSKIQNDK-NH_2) designed by substitution of amino acid tryptophan for the hydrophobic amino acids, glutamine and aspartic acid (Lee et al., 2002). Whereas, the AMP-P5 (KWKKLLKKPLLKKLLKKL-NH2) is analog of hybrid antimicrobial peptide CA-MA [Cecropin A (1–8)-Magainin 2 (1–12); KWKLFKK IGIGKFLHSAKKF-NH2] designed by flexible region (GIG→P)-substitution, Lys-(position 4, 8, 14, 15) and Leu- (positions 5, 6, 12, 13, 17, 17, 20) substitution (Park et al., 2006). The peptides were synthesized by a solid phase method using 9-fluorenylmethoxycarbonyl (Fmoc) chemistry.

In vitro antimicrobial activity of AMP-A3 (Lee et al., 2002) and P5 (Park et al., 2006) against various Gram-negative and Gram-positive bacterial strains were determined as minimum inhibitory concentrations (MIC). In vivo effects of AMP-A3 (Yoon et al., 2012) and P5 (Yoon et al., 2013) were also investigated in authors laboratory by supplementation of various levels of AMPs to weanling pigs diets.

2.2. Animals and experimental design

A total of 240 weanling pigs (Landrace × Yorkshire × Duroc; weaned at 21 d, average initial BW: 5.9 ± 0.56 kg) of both sexes were randomly allotted to four treatments on the basis of initial BW. There were four replicates in each treatment with 15 pigs per replicate. Dietary treatments were negative control (NC; basal diet without any antimicrobial growth promoters), positive control ((PC; basal diet + 150 mg/kg diet avilamycin (Elanco, Liverpool, UK)), basal diet with 60 mg/kg AMP-A3 (A3) and basal diet with 60 mg/kg AMP-P5 (P5). The levels of the AMPs used in the present study were based upon the results of our previous experiments (Yoon et al., 2012, 2013). To try to emulate the practical conditions the ZnO and acidifier (complex of lactic acid, formic acid and phosphoric acid at 1:1:1 ratio) were added to all the diets. The experimental diets were fed in two phases (phase I, d 0–14 and phase II, d 15–28). The diet for phase I was formulated to contain 14.28 MJ/kg ME and 15.5 g lysine/kg diet, whereas, the diet for phase II was formulated to contain 14.11 MJ/kg ME and 13.5 g lysine/kg diet (Table 1), and fed in meal form. All diets met or exceeded the nutrient requirements as suggested by NRC (1998; Table 2). Avilamycin and AMP's were added to basal the diet by equally replacing corn. For each of the AMP-containing experimental treatments, the AMP-A3 and AMP-P5 were mixed with carrier (corn) in such a way that addition of 6.0 g/kg to the basal diet would give 60 mg/kg diet of AMP-A3 and AMP-P5 respectively.

2.3. Experimental procedures and sampling

Pigs were weighed individually and feed consumption was measured at the end of each phase (d 14 and 28) to

Table 1
Ingredient and chemical composition of basal diet (as-fed basis).[a, b]

Item	Phase I (d 0–14)	Phase II (d 15–28)
Ingredients (g kg^{-1})		
Corn	–	340.8
Corn (expanded)	222.4	140.0
Corn starch	80.0	–
Soybean meal (44% CP)	–	191.0
Dehulled Soybean meal (47.5% CP)	121.1	150.0
Whey powder	150.0	70.0
Soy protein concentrate	80.0	–
Fish meal	20.0	20.0
Animal Fat	–	40.0
Lactose	110.0	–
Sucrose	30.0	10.0
Whey protein concentrate	60.0	–
Spray dried porcine plasma	45.0	–
Soy oil	45.0	–
Monocalcium phosphate	9.6	–
Dicalcium phosphate	–	11.5
Limestone	8.3	7.8
ZnO	3.0	3.0
Vitamin premix[c]	2.5	2.5
Mineral premix[d]	1.5	1.5
Salt	2.0	2.5
Acidifier[e]	2.0	1.5
L-Lysine (78%)	3.8	4.4
DL-Methionine (98%)	1.5	1.3
L-Threonine (98%)	1.3	1.2
Choline chloride (25%)	1.0	1.0
Chemical composition, calculated		
Metabolic energy (MJ/kg)	14.28	14.11
Crude protein (%)	22.84	21.70
Calcium (%)	0.80	0.77
Available phosphorus (%)	0.48	0.36
Lysine (%)	1.55	1.35

[a] Dietary treatments were NC: Negative control, diet without antimicrobial growth promoters; PC: Positive control, basal diet added with 150 mg avilamycin/kg diet; A3: basal diet added with 60 mg/kg diet antimicrobial peptide-A3 (AMP-A3); P5: basal diet added with 60 mg/kg diet antimicrobial peptide-P5 (AMP-P5). Avilamycin and AMP's were added to basal the diet by equally replacing corn.

[b] For each of the AMP-containing experimental treatments, the AMP's (AMP-A3 and AMP-P5) were mixed with carrier (corn) in such a way that addition of 6.0 g/kg would give 60 mg/kg diet AMP for treatment AMP-A3 and AMP-P5 respectively.

[c] Supplied per kg diet: 9600 IU vitamin A, 1800 IU vitamin D_3, 24 mg vitamin E, 1.5 mg vitamin B_1, 12 mg vitamin B_2, 2.4 mg vitamin B_6, 0.045 mg vitamin B_{12}, 1.5 mg vitamin K_3, 24 mg pantothenic acid, 45 mg niacin, 0.09 mg biotin, 0.75 mg folic acid, 18 mg ethoxyquin.

[d] Supplied per kg diet: 162 mg Fe (ferrous sulfate), 96 mg Cu (copper sulfate), 46.49 mg Mn (manganese sulfate), 0.9 mg I (calcium iodate), 0.9 mg Co (cobalt sulfate), 0.3 mg Se (sodium selenite).

[e] Complex of lactic acid, formic acid and phosphoric acid (1:1:1).

Table 2
Analyzed chemical composition of diets fed during digestibility study.[a,b]

Item	NC	PC	A3	P5
Phase I (d 8–14)				
Dry matter, g/kg	925.3	927.5	924.3	929.1
Crude protein, g/kg	217.7	218.9	219.7	214.2
Ash, g/kg	54.9	53.8	55.3	53.5
Calcium, g/kg	9.1	9.2	9.0	8.9
Total P, g/kg	5.2	5.0	4.9	5.1
Chromium, g/kg	2.3	2.3	2.2	2.2
Phase II (d 22–28)				
Dry matter, g/kg	924.7	926.6	929.3	925.7
Crude protein, g/kg	206.2	208.3	208.4	206.3
Ash, g/kg	52.8	54.1	53.6	53.3
Calcium, g/kg	8.8	9.1	9.3	8.9
Total P, g/kg	5.0	5.2	5.0	5.2
Chromium, g/kg	2.3	2.2	2.3	2.2
Essential amino acids (Phase II)				
Lysine	15.3	14.7	14.9	15.2
Arginine	13.2	11.7	11.5	12.3
Valine	8.8	9.5	9.7	9.2
Histidine	5.2	4.8	5.2	5.0
Leucine	20.1	20.8	19.9	20.3
Isoleucine	8.6	8.6	8.3	8.7
Metheonine	4.3	4.1	4.1	3.9
Phenylalanine	8.6	9.1	8.9	9.2
Threonine	10.2	9.8	10.3	9.7

[a] Dietary treatments were NC: Negative control, diet without antimicrobial growth promoters; PC: Positive control, basal diet added with 150 mg avilamycin/kg diet; A3: basal diet added with 60 mg/kg diet antimicrobial peptide-A3 (AMP-A3); P5: basal diet added with 60 mg/kg diet antimicrobial peptide-P5 (AMP-P5). Avilamycin and AMP's were added to basal the diet by equally replacing corn.

[b] For each of the AMP-containing experimental treatments, the AMP's (AMP-A3 and AMP-P5) were mixed with carrier (corn) in such a way that addition of 6.0 g/kg would give 60 mg/kg diet AMP for treatment AMP-A3 and AMP-P5 respectively.

calculate ADG, ADFI, and G: F. For the digestibility trial, diets containing 2.5 g chromium as indigestible marker/kg diet were fed to pigs during last seven days of each phase and then fecal grab samples were collected randomly from four pigs of each pen during last 3-d (3 samples from each pig) of each phase. About 100 g fecal samples were collected from each pig and feces collected over 3-d period were pooled to represent one pen. Feces were dried in an air forced drying oven at 60 °C for 72 h and ground in a Wiley laboratory mill (Thomas Model 4 Wiley® Mill, Thomas scientific, Swedesboro, NJ, USA) using a 1-mm screen and stored at −20 °C until further chemical analysis. Additionally, fresh fecal samples were collected from two random piglets per pen at d 28 and used for analysis of fecal microflora counts. The samples collected for microbial analysis were immediately placed on ice until the analyses was conducted later on the corresponding day. On d 14 and 28 of experiment, a 10-mL blood sample was collected by jugular vein puncture from two randomly selected pigs in each pen using a disposable vacutainer tube containing sodium heparin as an anticoagulant (Becton Dickinson, Franklin, NJ). After centrifugation (3000 × g for 15 min at 4 °C), serum samples were separated and stored at −20 °C and later analyzed for concentrations of immunoglobulins (IgG, IgA, and IgM).

To study the effects of dietary treatments on apparent ileal amino acid digestibility, small intestinal morphology and microflora of ileum and cecum digesta, two pigs per pen (reflecting the average body weight) were selected and sacrificed by electrocution on d 28 of experiment. The digesta (about 150 g/pig) from the terminal ileum (about 20 cm from ileo-ceacal junction) was collected and stored in two separate sterile plastic bottles per pigs for analysis of microflora and amino acids. The digesta samples collected for amino acid analysis were freeze-dried

until further analysis. The cecum digesta were also collected in sterilized plastic bottles and transported to laboratory on ice bath. The samples collected for microflora analysis were immediately placed on ice until analysis was conducted later on the same day. The samples of intestinal epithelium from the duodenum, jejunum and ileum after removal of digesta by flushing with physiological saline were submerged in a fixative solution (0.1 M collidine buffer, pH 7.3) containing 3% glutaraldehyde, 2% paraformaldehyde and 1.5% acrolein in plastic storage vials and then brought to laboratory to study morphological changes.

2.4. Chemical and microbial analyses

Experimental diets and excreta samples were analyzed in triplicate for DM (method 930.15; AOAC, 2007), CP (method 990.03; AOAC, 2007), ash (method 942.05; AOAC, 2007), Ca, and P (method 985.01; AOAC, 2007). The gross energy of diets and feces was measured by a bomb calorimeter (Model 1261, Parr Instrument Co., Moline, IL), while chromium was measured with an automated spectrophotometer (Jasco V-650, Jasco Corp., Tokyo, Japan) according to the procedure of Fenton and Fenton (1979). The amino acid compositions of feed samples were determined by HPLC (Waters 486, Waters Corp., Milford, MA) after acid hydrolysis. Methionine and cystine were determined following oxidation with performic acid (Moore, 1963). Serum IgG, IgA, and IgM were determined using radial immune-diffusion kit (Tripple J farm, Bellingham, WA, USA).

For analysis of microflora, 1 g of mixed digesta (ileum and cecum) or feces were diluted with 9 ml of Butterfields' phosphate buffer solution, followed by further serial dilutions in Butterfields' phosphate buffer dilution solution. Duplicate culture plates were then inoculated with 0.1 ml sample and incubated according to standard procedures for different bacteria. The total anaerobic bacteria were propagated in tryptic soy agar (Difco Laboratories, Detroit, MI, USA) under anaerobic conditions at 37 °C for 48 h; *Clostridium* species in tryptose sulphite cycloserine agar (Oxoid, Hampshire, UK) under anaerobic conditions at 37 °C for 48 h and coliforms in violet red bile agar (Difco Laboratories, Detroit, MI, USA) under aerobic conditions at 37 °C for 24 h. The anaerobic conditions during the assay of total anaerobic bacteria and *Clostridium* species were created by using gaspak anaerobic system (BBL, No. 260678, Difco, Detroit, MI, USA). The bacterial concentrations were transformed (log) before statistical analysis.

2.5. Small intestinal morphology

Three cross-sections for each intestinal sample were prepared after staining with azure A and eosin using standard paraffin embedding procedures (Uni et al., 1998). A total of 10 intact, well-oriented crypt-villus units were selected in triplicate for each intestinal cross-section. Villus height was measured from the tip of the villi to the villus crypt junction, crypt depth was defined as the depth of the invagination between adjacent villi and villus width was measured at the mid of the villus. All morphological measurements (villus height, crypt depth and villus width) were made in 10-μm increments by using an image processing and analysis system (Optimus software version 6.5, Media Cybergenetics, North Reading, MA).

2.6. Statistical analysis

Data generated in the present study were subjected to statistical analysis using the general linear model procedure of SAS (SAS Institute Inc., Cary, NC, USA). The one-way analysis of variance test was used for analysis of all parameters and when significant differences were determined among treatment means, they were separated by using Tukey's HSD tests. Pen was the experimental unit for analysis of growth performance and nutrient digestibility. For analysis of serum immunoglobulin, intestinal digesta, fecal microflora and intestinal morphology, the mean of two pigs from each pen was used as the experimental unit. Alpha level for determination of significance was 0.05.

3. Results

3.1. Growth performance

During phase I and phase II, pigs fed the PC and A3 diets had greater ($P < 0.05$) ADG than pigs fed the NC diet (Table 3). During phase I, the ADG of pigs fed the P5 diet was not different ($P > 0.05$) than pigs fed PC, P5 or NC diets; whereas, during phase II, the ADG of pigs fed the P5 diet was not different ($P > 0.05$) than pigs fed the NC and A3 diets. During phase II, the G:F of pigs fed PC, A3 and P5 diets was greater ($P < 0.05$) than pigs fed the NC diet. Moreover, pigs fed the PC diet had greater ($P < 0.05$) G:F than pigs fed the A3 and P5 diets. During the overall study

Table 3
Effects of dietary supplementation of antimicrobial peptides (AMP-A3 and AMP-P5) on growth performance of weanling pigs.[a]

Item	NC	PC	A3	P5	SEM	P-value
Phase I (d 0–14)						
ADG, g	238^{b}	259^{a}	253^{a}	249^{ab}	2.4	0.006
ADFI, g	344	364	358	357	3.0	0.121
G:F, g/kg	692	711	699	705	3.1	0.123
Phase II (d 15–28)						
ADG, g	369^{c}	395^{a}	385^{ab}	381^{bc}	2.9	0.001
ADFI, g	574	585	581	578	3.1	0.127
G:F, g/kg	642^{c}	676^{a}	658^{b}	663^{b}	3.4	0.001
Overall (d 0–28)						
ADG, g	303^{c}	327^{a}	319^{b}	315^{b}	2.3	0.001
ADFI, g	459	474	469	467	2.8	0.067
G:F, g/kg	**660^{c}**	**689^{a}**	**674^{b}**	**679^{ab}**	**2.9**	**0.001**

Values with different superscripts in the same row differ significantly ($P < 0.05$; $n = 4$).

[a] Dietary treatments were NC: Negative control, diet without antimicrobial growth promoters; PC: Positive control, basal diet added with 150 mg avilamycin/kg diet; A3: basal diet added with 60 mg/kg diet AMP-A3; P5: basal diet added with 60 mg/kg diet AMP-P5.

period, pigs fed PC, A3 and P5 diets had greater ($P < 0.05$) ADG and G:F than pigs fed the NC diet. The overall ADG of pigs fed the PC diet was greater ($P < 0.05$) than pigs fed the A3 and P5 diets. Also, the overall G:F of pigs fed the PC diet was greater ($P < 0.05$) than pigs fed the A3 diet; whereas, G:F of pigs fed the P5 diet was not different ($P > 0.05$) than pigs fed the PC or A3 diets. Dietary treatments had no effect ($P > 0.05$) on ADFI of pigs during phase I, phase II or overall study period.

Table 4
Effects of dietary supplementation of antimicrobial peptides (AMP-A3 and AMP-P5) on apparent total tract digestibility (%) of nutrients and apparent ileal digestibility (%) of essential amino acids in weanling pigs.[a]

Item	NC	PC	A3	P5	SEM	P-value
Phase I (d 8–14)						
Dry matter	82.9	85.2	83.8	84.5	0.32	0.062
Gross energy	80.9^{b}	84.6^{a}	83.2^{a}	83.9^{a}	0.41	0.001
Crude protein	79.9^{c}	83.1^{a}	81.4^{b}	81.7^{b}	0.33	0.001
Phase (d 22–28)						
Dry matter	80.1^{b}	84.2^{a}	82.3^{a}	83.4^{a}	0.48	0.001
Gross energy	79.2^{c}	83.2^{a}	81.2^{b}	81.3^{b}	0.42	0.002
Crude protein	77.9^{c}	81.1^{a}	79.6^{b}	79.7^{b}	0.32	0.001
Essential amino acids						
Lysine	71.3	74.3	72.5	73.2	0.67	0.497
Arginine	78.7	80.3	78.6	79.9	0.37	0.272
Valine	63.1	63.6	63.0	63.3	0.44	0.983
Histidine	73.2	74.1	73.5	73.7	0.49	0.917
Leucine	70.9	72.6	71.6	71.5	0.51	0.750
Isoleucine	69.1	69.9	69.7	69.5	0.62	0.980
Metheonine	75.7	77.7	77.3	76.8	0.46	0.428
Phenylalanine	70.8	74.2	74.9	74.6	0.83	0.281
Threonine	65.9	69.1	67.3	67.4	0.66	0.453

Values with different superscripts in the same row differ significantly (**$P < 0.05$; $n = 4$**).

[a] Dietary treatments were NC: Negative control, diet without antimicrobial growth promoters; PC: Positive control, basal diet added with 150 mg avilamycin/kg diet; A3: basal diet added with 60 mg/kg diet AMP-A3; P5: basal diet added with 60 mg/kg diet AMP-P5.

3.2. Apparent total tract digestibility

Pigs fed PC, A3 and P5 diets had greater ($P < 0.05$) ATTD of DM (d 28), GE and CP (phase I and II) than pigs fed the NC diet (Table 4). Moreover, pigs fed the PC diet had greater ($P < 0.05$) ATTD of CP (phase I and II) and GE (phase II) than pigs fed the A3 and P5 diets. However, dietary treatments had no effects ($P > 0.05$) on apparent ileal amino acid digestibility (Table 4).

3.3. Fecal and intestinal microbial population

Pigs fed the PC and P5 diets had fewer ($P < 0.05$) ileum and cecum total anaerobic bacteria, *Clostridium spp.* and coliforms than pigs fed the NC diet (Table 5). Also, pigs fed the PC and P5 diets had fewer ($P < 0.05$) ileum and cecum total anaerobic bacteria than pigs fed A3 diet. The ileal *Clostridium spp.* and coliforms and the cecal coliforms of pigs fed the A3 diet were not different ($P > 0.05$) from that of pigs fed PC, P5 and NC diets.

At d 28, pigs fed the PC, A3 and P5 diets had fewer ($P < 0.05$) fecal *Clostridium* spp. and coliforms than pigs fed the NC diet (Table 5). Additionally, pigs fed the PC diet had fewer fecal total anaerobic bacteria than pigs fed the NC and A3 diets, whereas the fecal total anaerobic bacteria of pigs fed the P5 diet was not different ($P > 0.05$) from that of pigs fed PC, NC or A3 diets.

3.4. Serum immunoglobulins

At d 14 and 28, dietary treatments had no effects ($P > 0.05$) on serum IgG, IgA and IgM concentrations. At d 14, average values of serum IgG, IgA and IgM were 7.88, 45.31 and 80.06 (mg/ml) respectively, whereas at d 28, average values of serum IgG, IgA and IgM were 7.96, 49.14 and 89.69 (mg/ml) respectively.

Table 5
Effects of dietary supplementation of antimicrobial peptides (AMP-A3 and AMP-P5) on bacterial populations (Log_{10} CFU/g) in feces, ileum and cecal content of weanling pigs (d 28).[a]

Item	NC	PC	A3	P5	SEM	P-value
Ileum						
Total anaerobic bacteria	8.64^{a}	8.17^{b}	8.56^{a}	8.26^{b}	0.04	0.017
Clostridium spp.	7.44^{a}	7.13^{b}	7.34^{ab}	7.21^{b}	0.03	0.008
Coliforms	4.42^{a}	4.11^{b}	4.29^{ab}	4.21^{b}	0.04	0.004
Cecum						
Total anaerobic bacteria	**8. 70^{a}**	**8.32^{b}**	**8.54^{a}**	**8.36^{b}**	**0.03**	**0.012**
Clostridium spp.	7.46^{a}	7.23^{b}	7.33^{b}	7.26^{b}	0.03	0.005
Coliforms	4.47^{a}	4.13^{b}	4.26^{ab}	4.17^{b}	0.04	0.006
Feces						
Total anaerobic bacteria	8.72^{a}	8.28^{b}	8.58^{a}	8.46^{ab}	0.05	0.005
Clostridium spp.	7.51^{a}	7.22^{b}	7.26^{b}	7.32^{b}	0.03	0.001
Coliforms	4.56^{a}	4.17^{c}	4.34^{b}	4.28^{b}	0.04	0.001

Values with different superscripts in the same row differ significantly (**$P < 0.05$; $n = 4$**).

[a] Dietary treatments were NC: Negative control, diet without antimicrobial growth promoters; PC: Positive control, basal diet added with 150 mg avilamycin/kg diet; A3: basal diet added with 60 mg/kg diet AMP-A3; P5: basal diet added with 60 mg/kg diet AMP-P5.

Table 6
Effects of dietary supplementation of antimicrobial peptides (AMP-A3 and AMP-P5) on small intestinal morphology in weanling pigs (d 28).[a]

Item	NC	PC	A3	P5	SEM[b]	P-value
Villus height, μm						
Duodenum	364[b]	417[a]	401[a]	408[a]	7.6	0.043
Jejunum	375[b]	425[a]	413[a]	419[a]	7.2	0.040
Ileum	362	380	368	387	6.1	0.498
Crypt depth, μm						
Duodenum	230	199	207	203	4.7	0.069
Jejunum	242	207	216	218	5.8	0.179
Ileum	236	227	233	227	5.2	0.927
VH:CD[b]						
Duodenum	1.6[b]	2.1[a]	1.9[a]	2.0[a]	0.06	0.002
Jejunum	1. 6[c]	2.1[a]	1.9[ab]	1.9[ab]	0.07	0.028
Ileum	1.6	1.7	1.6	1.7	0.05	0.718

Values with different superscripts in the same row differ significantly ($P < 0.05$; $n = 4$).

[a] Dietary treatments were NC: Negative control, diet without antimicrobial growth promoters; PC: Positive control, basal diet added with 150 mg avilamycin/kg diet; A3: basal diet added with 60 mg/kg diet AMP-A3; P5: basal diet added with 60 mg/kg diet AMP-P5.

[b] Villus height to crypt depth ratio.

3.5. Small intestinal morphology

Pigs fed the PC, A3 and P 5 diets had greater ($P < 0.05$) villus height and villus height: crypt depth (VH:CD) of the duodenum and jejunum than pigs fed the NC diet (Table 6). The VH:CD of the jejunum in pigs fed the PC diet was greater ($P < 0.05$) than that of pigs fed the A3 diet, whereas, VH:CD of the jejunum in pigs fed the P5 diet was not different ($P > 0.05$) than that of pigs fed the PC and A3 diets. Dietary treatments had no effects ($P > 0.05$) on villus height and VH:CD of the ileum and crypt depth of duodenum, jejunum and ileum.

4. Discussion

Antimicrobial peptides are a group of antimicrobials considered as novel alternatives to antibiotics due to their broad spectrum activity, safety and rare propensity for development of bacterial resistance (Bradshaw, 2003; Hancock and Lehrer, 1998). Positive effects of supplementation of antimicrobial peptides to weaning pigs diets have been reported previously (Tang et al., 2009; Wang et al., 2006; Yoon et al., 2012, 2013). The improved overall ADG and G:F of weanling pigs fed diet supplemented with antimicrobial peptides (AMP-A3 and P5) that was observed in the present study is in good agreement with Wang et al. (2011) who observed improvement in ADG and G:F of piglets fed diet supplemented with antimicrobial peptides. Similarly, it was reported that pigs fed diets supplemented with antimicrobial peptides isolated from the pig intestine (Bao et al., 2009), a potato antimicrobial peptide (Jin et al., 2008b) and lactoferricin-lactoferrampin (Tang et al., 2009) had improved growth performance compared with pigs fed diet without AMPs. In the present experiment, like in others (Jin et al., 2008b; Tang et al., 2009; Weber et al., 2001) weanling pigs fed diets containing antibiotics had greater overall growth performance. The improved ADG of weanling pigs fed diets supplemented with avilamycin and AMPs reported in the present study might be associated with improved feed efficiency, as is also evident by the greater digestibility of nutrients, reduced harmful intestinal microflora populations and improved intestinal morphology in pigs fed diets supplemented with avilamycin and AMPs. In the present study, although significance was reached in ADG and G:F of pigs fed AMPs and antibiotics, the magnitude of the difference was very small and therefore, according to these results, the impact of these effects might not be so big.

The greater ATTD of DM, CP and GE in weanling pigs fed diets supplemented with AMPs and avilamycin that was observed in the present study is in good agreement with data reported by Jin et al. (2008a) who observed an increase in ATTD of DM and CP in weanling pigs fed diets supplemented with an antimicrobial peptide obtained from *Solanum tuberosum*. In contrast to the report by Jin et al. (2008a) and present results, Jin et al. (2009) reported that there was no effect of the potato antimicrobial peptides on ATTD of DM and CP. This difference in results might be due to variation in level of dietary supplementation, origin of peptides (natural or synthetic) or mode of action of peptides. Similar to results of the present study, improved ATTD of nutrients in weanling pigs fed diets supplemented with the antibiotics have also been reported (Choi et al., 2011; Hu et al., 2008; Jin et al., 2008a). Improved ATTD of nutrients with antimicrobial feed additives are due to increased nutrient availability for absorption and piglets growth via suppression of growth and metabolic activities of harmful gut microflora with simultaneous alteration in intestinal morphology, intestinal epithelium thickness and epithelial cell turnover (Choi et al., 2011; Shim et al., 2010; Tang et al., 2009; Wang et al., 2006). In the present study, greater nutrient retention in pigs fed diets supplemented with antimicrobial peptides and antibiotics might be due reduction in number of harmful microflora (*Clostridium* spp. and coliforms) and improved intestinal morphology.

Antimicrobial peptide beneficially affect the host animal by improving its intestinal balance and creating gut micro-ecological conditions that suppress harmful microorganisms like *Clostridium* and coliforms and by favoring beneficial microorganisms like *Lactobacillus* and *Bifidobacterium* (Jin et al., 2008a, 2008b; Ohh et al., 2010; Tang et al., 2009; Wang et al., 2007). In this experiment, as in others (Jin et al., 2009, Ohh et al., 2010), weanling pigs fed diets containing antibiotics had fewer, total anaerobic bacteria, coliforms and *Clostridium spp*. count in feces and intestine. Dietary supplementation of the AMP-A3 and P5 also has potential for reducing harmful microflora like fecal and intestinal coliforms and *Clostridium spp.* in weanling pigs. Some of the previous studies also reported that dietary supplementation of antimicrobial peptides reduced fecal and intestinal coliforms and *Clostridium spp.* count (Jin et al., 2008a, 2008b; Ohh et al., 2010; Tang et al., 2009). In the present study, although significance were reached for total anaerobic bacteria, *Clostridium* spp. and coliforms in pigs fed diet supplemented with AMPs and antibiotics, the magnitude of the differences was very small and therefore, according to this

results, impact of these changes on improving microbial balance might not be so big.

An increased concentration of the serum immunoglobulins are required to regulate and enhance the immune functions, which provides health benefits, diminish weaning stress and improve health status and growth performance of weanling pigs (Turner et al., 2002). In the current study, supplementation of weanling pig diets with avilamycin or antimicrobial peptides had no effect on serum immunoglobulins concentrations. In contrast to results of present experiment, Shan et al. (2007) reported an increase in serum IgG, IgA, and IgM concentrations in weanling pigs fed diet supplemented with antimicrobial peptide (lactoferrin). However, similar to results of the present study, Shan et al. (2007) reported no effect of dietary supplementation of antibiotics on concentrations of serum IgG, IgA and IgM in weanling pigs.

In the current study, supplementation of the avilamycin and AMPs to weanling pig diets resulted in increased villus height and VH:CD of the duodenum and jejunum. Similar to results of the present study, Bao et al. (2009) reported increased villus height of the duodenum and jejunum in broiler chickens fed diets supplemented with antimicrobial peptides isolated from pig small intestine. Tang et al. (2009) observed that pigs fed diets supplemented with fusion peptide (lactoferricin-lactoferrampin) had increased villus height and villus height to crypt depth ratio of the jejunum and ileum. In contrast to report of Bao et al. (2009), Tang et al. (2009) and present results, Jin et al. (2008b) reported no effect of potato antimicrobial peptide on intestinal morphology of weanling pigs. This difference in results might be due to difference in species and origin (natural or synthetic) of antimicrobial peptides used. In general, intestinal morphology is indicative of gut health in pigs. Increasing the villus height suggests an increased surface area capable of greater absorption of available nutrients (Caspary, 1992). Increased villus height and villus height to crypt depth ratio are directly correlated with an increased epithelial turnover (Fan et al., 1997), and longer villi are correlated with activation of cell mitosis (Samanya and Yamauchi, 2002). In the present study, improved growth performance of pigs fed diets supplemented with avilamycin and AMPs might be due greater intestinal absorption due to increased villus height of the duodenum and jejunum and increased epithelial turnover of the nutrients due to increased VH:CD ratio of the duodenum and jejunum. The histomorphological changes in the intestine of weanling piglets reported in the present study provide new information regarding the potential for using AMPs (A3 and P5) in pigs feed as an antimicrobial growth promoters.

5. Conclusion

In conclusion, results obtained in the present study indicate that AMP-A3 and P5 have potential to improve the growth performance, nutrient digestibility, intestinal morphology and to reduce pathogenic bacteria in weanling pigs. The impact on several of the measured response parameters was quantitatively low, though. Therefore, further studies are needed to confirm the current results.

Conflict of interest

This work has no conflict of interest.

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