Research Article

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Effects of dietary supplementation with antimicrobial peptide-P5 on growth performance, apparent total tract digestibility, faecal and intestinal microflora and intestinal morphology of weanling pigs

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Abstract

BACKGROUND: The increase in drug-resistant bacteria and the ban on antibiotic growth promoters worldwide make the search for novel means of preventing bacterial infection and promoting growth performance imperative. In this sense, antimicrobial peptides are thought to be ideal candidates owing to their antimicrobial properties, broad spectrum of activity and low propensity for development of bacterial resistance. The aim of the present study was to investigate the effect of dietary supplementation with antimicrobial peptide-P5 (AMP-P5) on weanling pig nutrition.

RESULTS: A total of 240 weanling pigs were allotted to four treatments on the basis of initial body weight. There were four replicates in each treatment, with 15 pigs per replicate. Dietary treatments were negative control (NC, basal diet without antimicrobial), positive control (PC, basal diet + 1.5 g kg^{-1} apramycin), basal diet with 40 mg kg^{-1} AMP-P5 (P5-40) and basal diet with 60 mg kg^{-1} AMP-P5 (P5-60). Pigs fed the PC or P5-60 diet showed improved ($P < 0.05$) overall growth performance, apparent total tract digestibility of dry matter, crude protein and gross energy and reduced ($P < 0.05$) faecal and intestinal coliforms compared with pigs fed the NC diet.

CONCLUSION: The results obtained in this study indicate that dietary supplementation with 60 mg kg^{-1} AMP-P5 has the potential to improve the growth performance and apparent total tract digestibility of nutrients and reduce coliforms in weanling pigs.

Keywords: antimicrobial peptide-P5; apramycin; performance; weanling pig

INTRODUCTION

Since their discovery, antibiotics have been used in the animal feed industry as growth promoters or for treatment and prevention of infectious diseases. However, their continuous use/misuse has resulted in problems such as antibiotic residues in animal products, imbalance of normal microflora or reduction in beneficial intestinal bacterial population and generation of antibiotic-resistant bacteria.[1] Regulatory pressure and public perception of the need to remove antibiotics from animal feeds have made it necessary to identify alternatives to antibiotics in order to maintain growth performance benefits.[2] A number of research findings on the use of metabolically active substances as alternatives to antibiotic growth promoters have been documented.[2,3] In this sense, antimicrobial peptides (AMPs) are thought to be ideal candidates owing to their antimicrobial properties, broad spectrum of activity and low propensity for development of bacterial resistance.[4,5]

AMPs are small gene-encoded peptides that show a broad range of activity against Gram-positive and Gram-negative bacteria, fungi and mycobacteria.[6] They have been isolated and characterised from tissues and organisms representing virtually every kingdom and phylum. The use of AMPs as feed additives is still in its infancy, with most of the work being conducted *in vitro*, and

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very few animal studies have been documented. Wang *et al.*[7] reported improved growth performance, reduced pathogenic intestinal bacteria and enrichment of beneficial intestinal bacteria in weanling pigs fed diets supplemented with AMPs. Previous studies also reported positive effects of AMPs derived from potato on the performance of weanling pigs.[3,4] Hence the present study was undertaken with the objective of determining the effects of dietary supplementation with antimicrobial peptide-P5 (AMP-P5) on the growth performance, apparent total tract digestibility of nutrients and faecal and intestinal microflora of weanling pigs.

MATERIALS AND METHODS

The protocol for the present experiment was approved by the Institutional Animal Care and Use Committee of Kangwon National University, South Korea. The experiment was conducted at the facility of Kangwon National University farm.

Peptide synthesis

The antimicrobial peptide (AMP-P5) used in the present study was obtained from the Research Center for Proteineous Materials, Chosun University, Kwangju, South Korea. In brief, AMP-P5 (KWKKLLKKPLLKKLLKKL-NH_2) is a synthetic analogue of the hybrid antimicrobial peptide CA-MA (cecropin A(1–8)-magainin2(1–12): KWKLFKK IGIGKFLHSAKKF-NH_2) designed by flexible region (GIG $\rightarrow$ P) substitution and Lys-(positions 4, 8, 14, 15) and Leu-(positions 5, 6, 12, 13, 17, 17, 20) substitutions.[8] AMP-P5 was synthesised by a solid phase method using Fmoc (9-fluorenylmethoxycarbonyl) chemistry.[9] Peptide purification was carried out by preparative high-performance liquid chromatography on a C18 reverse phase column.

Animals, diets and feeding

A total of 240 weanling pigs (Landrace $\times$ Yorkshire $\times$ Duroc, average initial body weight 6.22 $\pm$ 0.28 kg, age 21 days) of mixed sex (120 male, 120 female) were randomly allotted to four treatments on the basis of body weight. There were four replicates in each treatment, with 15 pigs per replicate. All piglets were clinically healthy at the start of the trial and originated from 30 sows in their third parity. Dietary treatments were basal diet without antimicrobial (NC, negative control), basal diet supplemented with 1.5 g kg^{-1} apramycin (PC, positive control), basal diet supplemented with 40 mg kg^{-1} AMP-P5 (P5-40) and basal diet supplemented with 60 mg kg^{-1} AMP-P5 (P5-60). The two levels of AMP-P5 used in the present study were based on results of minimum inhibitory concentration.[8] The experimental diets were fed in mash form over two phases: phase I (days 0–14 post-weaning) and phase II (days 15–28 post-weaning). Diets for phase I (Table 1) were formulated to contain 14.28 MJ kg^{-1} metabolisable energy (ME) and 15.5 g kg^{-1} lysine. Diets for phase II (Table 1) were formulated to contain 14.11 MJ kg^{-1} ME and 13.5 g kg^{-1} lysine. All diets either met or exceeded the nutrient requirements (Table 2) recommended by the NRC.[10] The apramycin and AMP-P5 were added to the phase I and phase II diets at the expense of corn. For AMP-P5 supplementation the AMP-P5 was mixed with a carrier (corn) in such a way that addition of 4 and 6 g kg^{-1} to the diet would give 40 and 60 mg AMP-P5 kg^{-1} diet for treatments P5-40 and P5-60 respectively.

The piglets were housed in partially slatted concrete floor pens (2.8 m $\times$ 5 m). The temperature in the barn was 30 °C at the beginning of the experiment, was gradually decreased to 25 °C on day 8 and was then kept constant until the end of the experiment. Relative humidity ranged between 60 and 70%. Each pen was equipped with an infrared heating lamp as well as a self-feeder and low-pressure nipple drinker to allow *ad libitum* access to feed and water.

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

Item	Phase I (days 0–14 post-weaning)	Phase II (days 15–28 post-weaning)
Ingredients (g kg^{-1})		
Corn	–	340.8
Corn (Expanded)	222.4	140.0
Corn starch	80.0	–
Soya bean meal (440 g kg^{-1})	–	191.0
Soya bean meal (dehulled)	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 (780 g kg^{-1})	3.8	4.4
DL-Methionine (980 g kg^{-1})	1.5	1.3
L-Threonine (980 g kg^{-1})	1.3	1.2
Choline chloride (250 g kg^{-1})	1.0	1.0
Chemical composition, calculated		
ME (MJ kg^{-1})	14.28	14.11
CP (g kg^{-1})	228.4	217.0
Ca (g kg^{-1})	8.0	7.7
Av. P (g kg^{-1})	4.8	3.6
Lys (g kg^{-1})	15.5	13.5
TSAA[f] (g kg^{-1})	8.3	7.3

[a] Dietary treatments were: NC (negative control), basal diet without antimicrobial; PC (positive control), basal diet supplemented with 1.5 g kg^{-1} apramycin; P5-40, basal diet supplemented with 40 mg kg^{-1} AMP-P5; P5-60, basal diet supplemented with 60 mg kg^{-1} AMP-P5. Apramycin and AMP-P5 were added to the basal diet at the expense of corn.
[b] For AMP-P5 supplementation the AMP-P5 was mixed with a carrier (corn) in such a way that addition of 4 and 6 g kg^{-1} to the diet would give 40 and 60 mg AMP-P5 kg^{-1} diet for treatments P5-40 and P5-60 respectively.
[c] Supplied 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 and 18 mg ethoxyquin kg^{-1} diet.
[d] Supplied 162 mg Fe, 96 mg Cu, 46.49 mg Mn, 0.9 mg I, 0.9 mg Co and 0.3 mg Se kg^{-1} diet.
[e] Complex of lactic acid, formic acid and phosphoric acid.
[f] Total sulfur amino acids.

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

Item	NC	P5-40	P5-60	PC
Phase I (days 8–14 post-weaning)				
DM (g kg^{-1})	924.1	926.0	923.1	923.7
CP (g kg^{-1})	218.8	219.3	217.7	218.6
Ash (g kg^{-1})	51.1	51.9	49.8	50.9
Ca (g kg^{-1})	8.8	8.2	9.1	8.4
Total P (g kg^{-1})	5.4	5.7	5.8	5.4
Cr (g kg^{-1})	2.2	2.2	2.1	2.3
Phase II (days 22–28 post-weaning)				
DM (g kg^{-1})	928.8	930.1	927.9	928.4
CP (g kg^{-1})	202.7	202.0	203.1	201.2
Ash (g kg^{-1})	55.1	54.5	53.2	54.8
Ca (g kg^{-1})	9.2	9.0	9.3	9.0
Total P (g kg^{-1})	5.9	5.7	6.1	5.3
Cr (g kg^{-1})	2.3	2.3	2.2	2.3

[a] Dietary treatments were: NC (negative control), basal diet without antimicrobial; PC (positive control), basal diet supplemented with 1.5 g kg^{-1} apramycin; P5-40, basal diet supplemented with 40 mg kg^{-1} AMP-P5; P5-60, basal diet supplemented with 60 mg kg^{-1} AMP-P5. Apramycin and AMP-P5 were added to the basal diet at the expense of corn.
[b] Chromium (2.5 g kg^{-1}) was included in the diets from day 8 to day 14 (phase I) and from day 22 to day 28 (phase II).

Experimental procedures

The pigs were weighed individually at the start of the trial and on days 14 and 28. Feed that was not consumed was weighed at the end of each phase, and consumption was calculated for phase I (days 0–14), phase II (days 15–28) and the overall study period (days 0–28). Feed wastage was considered minimal, so feed disappearance was determined to be a reliable estimate of feed consumption. Feed consumption was calculated at the end of each phase, and the average daily feed intake (ADFI) and feed/gain ratio (F/G) were calculated. The average daily gain (ADG) and ADFI were calculated by dividing the total pen weight gain and total pen feed consumption respectively by the number of animal days (including body weight gain and feed intake of all dead piglets in the pen). The F/G for each pen was calculated by dividing the ADFI by the ADG. To evaluate the effect of dietary treatments on the apparent total tract digestibility (ATTD) of energy and nutrients, 2.5 g kg^{-1} Cr (as an inert, indigestible indicator) was included in the diets from day 8 to day 14 (phase I) and from day 22 to day 28 (phase II) as described by Hahn *et al*.[11] Faecal grab samples (100 g day^{-1} per pen) were collected from each pen on the last 3 days of each phase to determine the apparent nutrient retention. The faecal samples were pooled by pen, dried in a forced air drying oven at 60 °C for 72 h, ground in a Wiley laboratory mill (Thomas Model 4 Wiley® Mill, Thomas Scientific, Swedesboro, NJ, USA) with a 1 mm screen and used for chemical analysis. Additionally, fresh faecal samples were collected from two pigs in each pen on days 14 and 28 and used for measuring faecal bacterial counts. The samples collected for microbial analysis were immediately placed on ice (2–3 h) and transported to the laboratory for further analysis on the same day. To study the effect of dietary treatments on the microflora of ileum and caecum digesta, two representative pigs (one male, one female) from each pen reflecting the average body weight were selected and sacrificed by electrocution on day 28. The digesta from the ileum and caecum were collected separately in sterile plastic bottles for microbial analysis. The samples collected for microbial analysis were immediately placed on ice until analyses were conducted later on the same day.

Chemical and microbial analyses

Dry matter (DM), crude protein (CP) and ash analyses of experimental diets and excreta samples were done according to AOAC methods.[12] The Ca and P contents of diets and excreta samples were measured according to AOAC method 7.099b.[13] Gross energy (GE) was measured with a bomb calorimeter (Model 1261, Parr Instrument Co., Moline, IL, USA), and the Cr concentration in experimental diets and excreta samples was determined with an automated spectrophotometer (Jasco V-650, Jasco Corp., Tokyo, Japan) according to the procedure of Fenton and Fenton.[14] The ATTD of nutrients was calculated using the formula

$$\text{ATTD (g kg}^{-1}) = [1 - (N_f \times C_d)/(N_d \times C_f)] \times 1000$$

where N_f is the nutrient concentration in the faeces (g kg^{-1} DM), N_d is the nutrient concentration in the diet (g kg^{-1} DM), C_f is the Cr concentration in the faeces (g kg^{-1} DM) and C_d is the Cr concentration in the diet (g kg^{-1} DM)

The microbiological assay of faecal samples and intestinal chyme was carried out according to the procedure of Torrallardona *et al*.[15] The microbial groups enumerated were total anaerobic bacteria (TAB; plate count agar, Difco Laboratories, Detroit, MI, USA), coliforms (violet red bile agar, Difco Laboratories) and *Clostridium* spp. (tryptose sulfite cycloserine agar, Oxoid, Basingstoke, UK). The anaerobic conditions during the assay of TAB and *Clostridium* spp. were created using a GasPak anaerobic system (BBL No. 260 678, Difco Laboratories).

Statistical analysis

All data obtained in the current study were analysed in accordance with a randomised complete block design using the general linear model procedure of SAS (SAS Institute Inc., Cary, NC, USA). One-way analysis of variance was applied to all parameters. When significant differences were observed among treatment means, they were separated by Tukey's honest significant difference test. Pen was the experimental unit for analysis of all parameters. The bacterial concentrations were logarithmically transformed before statistical analysis. Comparisons with $P < 0.05$ were considered significant.

RESULTS

Growth performance

Two piglets from the NC group and one piglet from the PC group died owing to diarrhoea during phase I of the experiment. For these piglets, missing values were calculated for this period. All other piglets remained in good health throughout the experimental period. During phase I, phase II and the overall study period, pigs fed the PC or P5-60 diet had greater ($P < 0.05$) ADG than pigs fed the NC diet (Table 3). The ADG (phase I and phase II) of pigs fed the P5-40 diet was not different from that of pigs fed the P5-60 or NC diet. During phase I, pigs fed the PC or P5-60 diet had higher ADFI than pigs fed the NC diet. However, the ADFI of pigs fed the P5-40 diet was not different from that of pigs fed the PC, P5-60 or NC diet. During phase II and the overall study period, pigs fed the PC or P5-60 diet had lower ($P < 0.05$) F/G than pigs fed the NC diet. The F/G of pigs fed the P5-40 diet was not different from that of pigs fed the P5-60 or NC diet.

Table 3. Effects of AMP-P5 on growth performance of weanling pigs[a]

Item	NC	P5-40	P5-60	PC	SEM[b]	*P*
Phase I (days 0–14)						
ADG (g)	275c	282bc	302ab	308a	4.36	0.004
ADFI (g)	419b	426ab	438a	442a	2.98	0.008
F/G	1.53	1.51	1.45	1.43	0.02	0.099
Phase II (days 15–28)						
ADG (g)	352c	367bc	392ab	401a	6.06	0.002
ADFI (g)	593	600	601	609	2.26	0.089
F/G	1.68a	1.64ab	1.53b	1.52b	0.02	0.005
Overall (days 0–28)						
ADG (g)	311c	323c	335ab	357a	5.61	0.006
ADFI (g)	506	513	519	525	3.22	0.157
F/G	1.63a	1.60ab	1.55bc	1.48c	0.02	0.001

Means within a row without a common letter are significantly different ($P < 0.05$).
[a] Dietary treatments were: NC (negative control), basal diet without antimicrobial; PC (positive control), basal diet supplemented with 1.5 g kg^{-1} apramycin; P5-40, basal diet supplemented with 40 mg kg^{-1} AMP-P5; P5-60, basal diet supplemented with 60 mg kg^{-1} AMP-P5.
[b] Standard error of means.

Table 4. Effects of AMP-P5 on apparent total tract digestibility (g kg^{-1}) of weanling pigs[a]

Item	NC	P5-40	P5-60	PC	SEM[b]	*P*
Day 14						
DM	806c	818bc	834ab	844a	4.29	0.001
CP	797c	806bc	817ab	826a	3.44	0.002
GE	789	795	808	825	6.83	0.293
Ash	493	503	523	528	6.93	0.155
Ca	458	454	478	484	6.80	0.331
P	394	389	414	428	6.94	0.161
Day 28						
DM	817b	819b	826ab	838a	2.57	0.005
CP	781b	792b	808ab	824a	5.24	0.006
GE	795b	805ab	815a	828a	5.01	0.005
Ash	507	504	511	518	6.86	0.924
Ca	448	456	458	472	6.30	0.656
P	424	424	426	436	4.93	0.808

Means within a row without a common letter are significantly different ($P < 0.05$).
[a] Dietary treatments were: NC (negative control), basal diet without antimicrobial; PC (positive control), basal diet supplemented with 1.5 g kg^{-1} apramycin; P5-40, basal diet supplemented with 40 mg kg^{-1} AMP-P5; P5-60, basal diet supplemented with 60 mg kg^{-1} AMP-P5.
[b] Standard error of means.

Apparent total tract digestibility of nutrients

During phase I, pigs fed the PC or P5-60 diet had higher ($P < 0.05$) ATTDs of DM and CP than pigs fed the NC diet (Table 4). The ATTDs of CP and DM in pigs fed the P5-40 diet were not different from those in pigs fed the NC or P5-60 diet. During phase II, pigs fed the PC or P5-60 diet had greater ($P < 0.05$) ATTDs of DM, CP and GE than pigs fed the NC diet, whereas the ATTDs of all these nutrients in pigs fed the P5-40 diet were not different from those in pigs fed the NC or P5-60 diet. Also, the ATTDs of DM and CP were not different between the PC and P5-60 treatments during phase I or phase II. Dietary treatments had no effect on the ATTDs of ash, Ca and P.

Table 5. Effects of AMP-P5 on bacterial populations (log_{10} colony-forming units g^{-1}) in faeces of weanling pigs[a]

Item	NC	P5-40	P5-60	PC	SEM[b]	*P*
Day 14						
Total anaerobic bacteria	8.75	8.73	8.62	8.56	0.04	0.315
Clostridium spp.	7.43	7.35	7.24	7.18	0.06	0.557
Coliforms	6.78a	6.62ab	6.51bc	6.37c	0.05	0.001
Day 28						
Total anaerobic bacteria	8.61	8.59	8.49	8.45	0.05	0.623
Clostridium spp.	7.68	7.57	7.45	7.31	0.06	0.085
Coliforms	6.45a	6.37ab	6.30b	6.27b	0.05	0.015

Means within a row without a common letter are significantly different ($P < 0.05$).
[a] Dietary treatments were: NC (negative control), basal diet without antimicrobial; PC (positive control), basal diet supplemented with 1.5 g kg^{-1} apramycin; P5-40, basal diet supplemented with 40 mg kg^{-1} AMP-P5; P5-60, basal diet supplemented with 60 mg kg^{-1} AMP-P5.
[b] Standard error of means.

Table 6. Effects of AMP-P5 on bacterial populations (log_{10} colony-forming units g^{-1}) in ileal and caecal contents of weanling pigs (day 28)[a]

Item	NC	P5-40	P5-60	PC	SEM[b]	*P*
Ileum						
Total anaerobic bacteria	8.48	8.44	8.26	8.19	0.05	0.319
Clostridium spp.	7.25	7.08	6.99	6.92	0.06	0.201
Coliforms	6.17a	5.98b	5.85bc	5.77c	0.05	0.001
Caecum						
Total anaerobic bacteria	8.57a	8.42ab	8.36ab	8.28b	0.04	0.023
Clostridium spp.	7.30	7.12	7.04	6.95	0.06	0.108
Coliforms	6.63a	6.51ab	6.45b	6.41b	0.04	0.014

Means within a row without a common letter are significantly different ($P < 0.05$).
[a] Dietary treatments were: NC (negative control), basal diet without antimicrobial; PC (positive control), basal diet supplemented with 1.5 g kg^{-1} apramycin; P5-40, basal diet supplemented with 40 mg kg^{-1} AMP-P5; P5-60, basal diet supplemented with 60 mg kg^{-1} AMP-P5.
[b] Standard error of means.

Faecal and intestinal microbial populations

Pigs fed the PC or P5-60 diet had lower ($P < 0.05$) faecal coliform concentration (days 14 and 28) than pigs fed the NC diet (Table 5). The faecal coliform concentration in pigs fed the P5-40 diet was not different from that in pigs fed the NC or P5-60 diet (days 14 and 28) or the PC diet (day 28). At days 14 and 28 the coliform concentrations of treatments PC and P5-60 were not different. Dietary treatments had no effect on faecal TAB and *Clostridium* spp. concentrations.

Intestinal microbial populations were analysed on day 28. Dietary treatments had no effect on ileal TAB and *Clostridium* spp. concentrations (Table 6). However, pigs fed the PC, P5-40 or P5-60 diet had lower ($P < 0.05$) ileal coliform concentration than pigs fed the NC diet. The ileal coliform concentrations in pigs fed the PC and P5-60 diets were not different. The caecal coliform

concentration in pigs fed the PC or P5-60 diet was lower ($P < 0.05$) than that in pigs fed the NC diet, whereas the caecal coliform concentration in pigs fed the P5-40 diet was not different from that in pigs fed the NC, PC or P5-60 diet. Pigs fed the PC diet had lower ($P < 0.05$) caecal TAB concentration than pigs fed the NC diet, while the caecal TAB concentration in pigs fed the P5-40 or P5-60 diet was not different from that in pigs fed the NC or PC diet.

DISCUSSION

The mode of action of AMPs is based on structural properties that govern their interaction with target cells.[16] The antimicrobial activity of AMPs can be improved by designing analogue peptides by modifying the structural properties of natural peptides.[17–19] In the present study we used the novel analogue peptide AMP-P5,[8] synthesised by chain length deletion and increasing the net positive charge and hydrophobicity of the hybrid peptide CA-MA, and its effects on the growth performance, nutrient retention and faecal and intestinal microflora of weanling pigs were evaluated when used as a substitute for antibiotic.

The improved growth performance of weanling pigs fed diets supplemented with AMP-P5 observed in the present experiment is in good agreement with data reported by Wang *et al*.,[5] who observed an improvement in ADG and F/G of weanling pigs fed diets supplemented with an AMP. Jin *et al*.[3] reported a linear improvement in growth performance of weanling pigs fed diets supplemented with AMPs derived from potato (*Solanum tuberosum*). Other studies on weanling pigs fed diets supplemented with AMPs also observed an improvement in ADG.[20,21] Similar to the present findings, previous studies[4,22,23] on antibiotic supplementation of weanling pig diets showed greater overall growth performance compared with pigs fed control diets. The improved ADG of weanling pigs fed diets supplemented with antibiotic and P5-60 found in the present study might be associated with greater feed intake or improved feed efficiency, as is also evident by the greater digestibility of DM, GE or CP and reduced population of coliforms in weanling pigs fed the antibiotic- or P5-60-supplemented diet.

In the present study, supplementation of weanling pig diets with AMP-P5 (60 mg kg^{-1} diet) and apramycin improved the ATTDs of DM, CP and GE. These findings are consistent with studies of Jin *et al*.,[3,4] who reported greater nutrient retention in pigs fed diets supplemented with apramycin or with AMPs derived from potato. The greater nutrient retention in pigs fed diets supplemented with AMP-P5 might be due to modulation of the gut environment, improved gut barrier function via competitive exclusion of pathogenic micro-organisms, improvement in beneficial intestinal microbial balance or stimulation of the mucosal immune system.[3,4,24] On the other hand, the improved nutrient retention in pigs fed diets supplemented with apramycin might be due to increased nutrient availability for absorption or piglet 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.[25,26] Improved digestibility of nutrients in weanling pigs fed apramycin was also observed in some previous studies,[4,26,27] and this improved performance might be due to the benefits obtained from the antibacterial properties of apramycin. In our study the ATTDs of DM, CP and GE in weanling pigs fed diets supplemented with apramycin and P5-60 are comparable, which indicates the potential of AMP-P5 as an antimicrobial in weanling pig diets.

In this study, AMP-P5 (P5-60) and apramycin showed potential in reducing harmful microflora such as faecal and intestinal coliforms and caecal *Clostridium* spp. in weanling pigs. Previous studies on pigs fed diets supplemented with other antibacterial peptides also observed reductions in faecal or intestinal coliforms and *Clostridium* spp.[3,4,24,28] AMPs beneficially affect the host animal by improving its intestinal microbial balance and creating gut micro-ecological conditions that suppress harmful micro-organisms such as *Clostridium* and coliforms and by favouring beneficial micro-organisms such as *Lactobacillus* and *Bifidobacterium*.[3,4,7,24,28] Our results suggest that P5-60 has the potential to improve the faecal and intestinal microbial balance of weanling pigs and can be used as an alternative to antibiotics.

In the present experiment the growth performance, ATTDs of energy and nutrients and microflora populations of weanling pigs fed diets supplemented with P5-40 (40 mg AMP-P5 kg^{-1} diet) were not statistically different from those of pigs fed non-supplemented diets. Our observations indicate that dietary supplementation with only 40 mg kg^{-1} AMP-P5 is insufficient to have significant effects on the nutritional performance of weanling pigs, even though it was effective *in vitro*. This variation in results might be due to variation in conditions of *in vitro* and *in vivo* studies, partial inactivation or degradation of AMP-P5 in the gastrointestinal tract of pigs and some unidentified factors. However, in the present study, no differences were observed among pigs fed diets supplemented with antibiotic and P5-60 for any of the measured parameters, which indicates the potential of 60 mg AMP-P5 kg^{-1} diet as a novel alternative to antibiotic growth promoters. Nevertheless, we used only four replicates per treatment, with 15 pigs in each replicate, which is not sufficient to conclude that P5-60 can be used as an alternative to the antibiotic apramycin. Therefore further studies are required with increased numbers of pigs and replicates to confirm the potential of AMP-P5 as an alternative to antibiotic growth promoters.

CONCLUSIONS

The results obtained in the present experiment indicate that dietary supplementation with 60 mg kg^{-1} AMP-P5 has the potential to improve the growth performance and nutrient digestibility and reduce pathogenic bacteria in weanling pigs. However, further studies are needed to identify the mechanism of action of AMP-P5.

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