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Effects of dietary supplementation with an antimicrobial peptide-P5 on growth performance, nutrient retention, excreta and intestinal microflora and intestinal morphology of broilers

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ABSTRACT

The present study investigated the effects of dietary supplementation of antimicrobial peptide-P5 (AMP-P5) on growth performance, nutrient retention, excreta and intestinal digesta microflora and intestinal morphology of broilers. Three hundred twenty-d-old chicks (Ross 308) were randomly allotted to 4 dietary treatments, each consisting of 4 pens as replicates, with 20 chicks in each pen. The dietary treatments were: NC (negative control; basal diet), PC (positive control; basal diet supplemented with 15 mg avilamycin/kg diet) and AMP-P5 (basal diet supplemented with 40 and 60 mg AMP-P5/kg diets). The experimental diets were fed in 2 phases, starter (d 0–21) and finisher (d 22–35). The overall (d 0–35) body weight (BW) gain and retention of dry matter (DM) and nitrogen (N; d 20–21 and d 34–35) were greater ($P<0.05$) in birds fed the PC and 60 mg AMP-P5/kg diets than birds fed the NC diet. Moreover, increasing levels of dietary AMP-P5 linearly ($P<0.05$) improved BW gain (starter, finisher and overall), feed conversion ratio (FCR; starter) and retention of DM and N (d 20–21 and d 34–35). The population of excreta of total anaerobic bacteria (TAB, d 35) and coliforms (d 21 and d 35) and the ileal and cecal digesta coliforms (d 35) was fewer ($P<0.05$) in birds fed the PC and 60 mg AMP-P5/kg diets compared with birds fed the NC diet. The villus height of the duodenum and jejunum and villus height:crypt depth (VH:CD) of the duodenum, jejunum and ileum were greater ($P<0.05$) in the birds fed the PC and 60 mg AMP-P5/kg diets than birds fed the NC diet. Increasing levels of dietary AMP-P5 linearly ($P<0.05$) reduced ($P<0.05$) excreta TAB (d 35), coliforms (d 21 and d 35) and the coliforms in the ileal and cecal digesta (d 35). Moreover, increasing levels of dietary AMP-P5 linearly increased ($P<0.05$) villus height (duodenum and jejunum) and VH:CD (duodenum, jejunum and ileum) and linear reduction ($P<0.05$) in crypt depth of the jejunum. These results indicate that dietary supplementation with 60 mg AMP-P5/kg has the potential to improve the growth performance, nutrient retention, intestinal morphology and reduce intestinal and excreta coliforms in broilers.

Abbreviations: AMP, antimicrobial peptide; BW, body weight; CFU, colony forming unit; DM, dry matter; FCR, feed conversion ratio; GE, gross energy; ME, metabolic energy; N, nitrogen; NC, negative control; PC, positive control; TAB, total anaerobic bacteria; VH:CD, villus height:crypt depth.

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1. Introduction

The increase in drug-resistant bacteria and the ban on the antibiotic growth promoters in some developed countries make the search for a novel means for preventing bacterial infection and promoting growth performance (Van den Bogaard and Stobberingh, 2000). A number of research findings on the use of metabolically active substances as alternatives to the antibiotic growth promoters have been reported (Bae et al., 1999; Bao et al., 2009; Choi et al., 2011; Yoon et al., 2012, 2013). In this context, synthetic analogs of the natural antimicrobial peptides (AMPs) are ideal candidates, due to their antimicrobial properties, broad spectrum activity, speed of action and a low propensity for the development of bacterial resistance (Hancock and Lehrer, 1998; Bradshaw, 2003).

The AMPs have been found in a variety of sources from prokaryotes to higher eukaryotes and constitute a part of the first line of host defense (Ganz, 2003; Maróti et al., 2011). The AMPs 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 rapid and broad spectrum properties and a low propensity for the development of bacterial resistance, the AMPs have been proposed as antimicrobials to treat microbial infections, particularly those caused by antibiotic resistant bacteria (Hadley and Hancock, 2010). In some studies, it has been reported that dietary supplementation of different AMPs to broiler diets improved the growth performance (Wang et al., 2009; Ohh et al., 2010), villus height of duodenum and jejunum (Bao et al., 2009), mucosal immunity (Liu et al., 2008) and reduced population of harmful intestinal microflora (Ohh et al., 2009). Recent study in our laboratory reported improved growth performance, nutrient retention and intestinal microflora in weanling pigs fed diet supplemented with AMP-P5 (Yoon et al., 2012). Hence, the present study was undertaken to determine the effects of dietary supplementation of the synthetic antimicrobial peptide (AMP-P5) on growth performance, nutrient retention, excreta and intestinal microflora, and intestinal morphology of broilers.

2. Materials and methods

2.1. Peptide synthesis

The AMP-P5 used in present study was obtained from the Research Center for Proteineous Materials, Chosun University, Kwangju, Republic of Korea. In short, AMP-P5 (KWKKLLKKPLLKKLLKKL-NH_2) is analog of hybrid antimicrobial peptide CA-MA [cecropin A (1-8)-magainin 2(1-12):KWKLFKK IGIGKFLHSAKKF-NH_2] designed by flexible region (GIG→P)-substitution, Lys-(position 4, 8, 14, 15) and Leu-(positions 5, 6, 12, 13, 17, 20) substitution (Park et al., 2006). The AMP-P5 was synthesized by solid phase method using Fmoc (9-fluorenyl-methoxycarbonyl)-chemistry (Merrifield, 1986). Peptide purification was carried out by preparative HPLC on C_{18} reverse phase column.

2.2. Birds, diets and management

Three hundred twenty-d-old chicks (Ross 308) were randomly allotted to 4 dietary treatments, each consisting of 4 pens as replicates, with 20 chicks in each pen. The dietary treatments were: negative control (NC; basal diet), positive control (PC; basal diet supplemented with 15 mg/kg diet avilamycin (Elanco, Liverpool, UK)), and AMP-P5 (basal diet added with 40 and 60 mg AMP-P5/kg diet). The NC (diet without antimicrobials) was considered as 0 mg AMP-P5/kg diet. Two levels of the AMP-P5 used in present study were based upon results of dietary supplementation of AMP-P5 to weanling pigs (Yoon et al., 2012). Basal diet was in mash form and was formulated for starter (d 0–21) and finisher (d 22–35) periods (Table 1). All the nutrients met or exceeded the nutrient requirements as recommended by NRC (1994). The avilamycin and the AMP-P5 were added to basal diet at the expense of corn.

The experiment was conducted according to the guidelines of the Institutional Animal Care and Use Committee of Kangwon National University, Chuncheon, Republic of Korea. The birds were housed in rice hull-covered floor pens. Each pen was provided with a self-feeder and hanging bell drinker to allow free access to feed and water. The house temperature was maintained at 34 °C for the first 5 d and then gradually reduced according to normal management practices, until a temperature of 23 °C was achieved. A 23-h light:1-h dark cycle was maintained.

2.3. Sample preparation and measurements

The birds were individually weighed at the start of the trial and on d 21 and d 35. Body weight (BW) gain, feed intake and feed conversion ratio (FCR; pen based) were calculated for starter (d 0–21), finisher (d 21–35) and overall study period (d 0–35). The BW gain and the feed intake were calculated by dividing total pen weight gain and total pen feed consumption by the number of bird days (including body weight gain and feed intake of all dead birds in the pen). The FCR for each pen was calculated by dividing the feed intake by the BW gain. Two balance trials were conducted during the last week of each phase to determine retention of DM and N. From d 14 (starter) and d 28 (finisher) onward, 2 birds from each replicate were allocated individual cages (one bird/cage) to facilitate collection of excreta samples. The starter and finisher diets containing 2.5 g/kg chromium as an indigestible marker were fed from d 15 and 28 onward, respectively. Excreta samples (about 100 g/d per bird) were collected from each bird for last 3 d of each phase. The excreta samples collected for 3 d were pooled and dried in a forced air drying oven at 60 °C for 72 h, and ground in a Wiley laboratory mill (Thomas Model 4 Wiley® Mill,

Table 1
Ingredient and chemical composition of basal diets (as-fed basis).

Item	Starter (d 0–21)	Finisher (d 22–35)
Ingredients (g/kg)		
Corn	556.8	583.4
SBM (440 g crude protein/kg)	261.9	214.3
Wheat	20.0	50.0
Corn gluten meal	70.0	80.0
Fish meal (550 g crude protein/kg)	20.0	–
Animal fat	–	38.6
Soy-oil	36.5	–
Dicalcium phosphate	18.4	16.6
Limestone	7.5	7.5
Salt	3.0	3.0
L-Lysine (780 g/kg)	1.1	2.2
DL-Methionine (500 g/kg)	1.8	0.3
Choline chloride (500 g/kg)	1.0	1.3
Vitamin premix[a]	1.0	1.3
Trace mineral premix[b]	1.0	1.5
Analyzed chemical composition unless indicated otherwise		
ME (MJ/kg; calculated)[c]	13.40	13.40
CP (g/kg)	218.9	196.7
Ca (g/kg)	9.3	9.7
Avail. P (g/kg)	4.6	4.1
Lysine (g/kg)	11.6	10.1
Methionine (g/kg)	5.2	4.0
Met + Cys (g/kg)	8.8	7.2

[a] Provides (per kg diet): vitamin A palmitate, 9000 IU; cholecalciferol, 1800 IU; DL-α-tocopheryl acetate, 30 mg; menadione, 1 mg; thiamin, 1 mg; riboflavin, 10 mg; pyridoxine, 4 mg; cyanocobalamin, 0.02 mg; niacin, 30 mg; pantothenic acid, 12 mg; folic acid, 0.5 mg; biotin, 0.2 mg.
[b] Provides (per kg diet): 80 mg Fe (ferrous sulfate), 6 mg Cu (copper sulfate), 70 mg Zn (zinc sulfate), 84 mg Mn (manganese sulfate), 1.4 mg I (calcium iodate), 0.07 mg Co (cobalt sulfate), 0.2 mg Se (sodium selenite).
[c] Based on NRC (1994) values.

Thomas Scientific, Swedesboro, NJ, USA) using a 1-mm screen and used for chemical analysis. The nutrient retention (%) was calculated as: nutrient retention (%) = 100 − [100 × (% Cr in feed/% Cr in excreta) × (% nutrient in excreta/% nutrient in feed)]. Additionally, fresh excreta samples were collected from each bird housed in individual cages on d 21 and d 35 and used for measuring the excreta 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.

At the end of the experimental feeding (d 35) 8 birds per treatment (2 birds from each pen) were slaughtered (stunning, bleeding, scalding, plucking, evisceration) to study the intestinal morphology and bacteria of the ileal and cecal digesta. The birds were fasted for 6 h before the slaughter for emptying the crop before slaughter to prevent the possibility of the excretion of crop digesta. The samples of the fresh excreta, ileal and cecal digesta were stored on ice until analyzed on the same day. For study of intestinal morphology, samples of intestinal segments were also collected from the region of duodenum (the midpoint), jejunum (distal to mid point) and ileum (10 cm proximal to the ileocecal junction) and after removal of its contents flushed with physiological saline and submerged in a fixative solution (0.1 M collidine buffer, pH 7.3) containing 3% glutaraldehyde, 2% para-formaldehyde and 1.5% acrolein as described earlier (Choi et al., 2011).

2.4. Chemical and microbial analysis

Dry matter and CP analyses of the experimental diets and excreta samples were carried out as described earlier (AOAC, 2007). The chromium concentration in experimental diets and excreta samples was determined with an atomic absorption spectrophotometer (Model AA-680G, Shimadzu, Kyoto, Japan) according to the procedure of Fenton and Fenton (1979). Methionine and cystine were determined after oxidation with performic acid (Moore, 1963). The excreta, ileal and cecal digesta microflora were analyzed according to the procedure of Jin et al. (2009). The microbial groups analyzed were total anaerobic bacteria (TAB; plate count agar, Difco Laboratories, Detroit, MI), *Clostridium* spp. (tryptose sulphite cycloserine agar, Oxoid, Hampshire, UK), and coliforms (violet red bile agar, Difco Laboratories, Detroit, MI). The microbial populations were log transformed before statistical analysis.

2.5. Intestinal morphology

The intestinal morphology was analyzed according to the procedure of Yoon et al. (2012). In short, three cross-sections for each intestinal sample were prepared after staining with azure A and eosin using standard paraffin embedding procedures. A total of 10 intact, well-oriented crypt-villus units were selected in triplicate for each intestinal cross-section. The villus height was measured from the tip of the villus to the villus crypt junction, and crypt depth was defined as the depth of the invagination between adjacent villi. All morphological measurements (villus height and crypt depth) 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. In addition, orthogonal polynomial contrasts were used to determine significant linear and quadratic response to increasing dietary AMP-P5 levels (0, 40 and 60 mg AMP-P5/kg diet). Comparisons with P<0.05 were considered significant.

3. Results

3.1. Growth performance

The BW gain, feed intake and FCR of broilers during various phases of production are presented in Table 2. The BW gain of birds fed the PC and 60 mg AMP-P5/kg starter and finisher diets was greater (P<0.05) when compared with birds fed the NC diet. The FCR was only affected in the starter phase that was better in the PC-fed diet compared with the NC diet. Moreover, the BW gain (starter and finisher) and FCR (starter) were linearly improved (P<0.05) as the level of dietary AMP-P5 was increased. The overall BW gain of birds fed the PC and 40 and 60 mg AMP-P5/kg diets was greater (P<0.05) than birds fed the NC diet, whereas the overall feed intake and FCR were not different among dietary treatments. Also, the overall BW gain increased (linear, P<0.05) with increase in dietary AMP-P5.

3.2. Nutrient retention

On d 20–21 and d 34–35, the retention of DM and N were greater (P<0.05; Table 3) in birds fed the PC and 60 mg AMP-P5/kg diets than birds fed the NC diet. The retention of DM and N in birds fed the 60 mg AMP-P5/kg diet was not different from birds fed the PC and 40 mg AMP-P5/kg diets. Moreover, the retention of DM and N (d 20–21 and d 34–35) were linearly increased (P<0.05) with increase in dietary AMP-P5.

3.3. Excreta and intestinal microbial population

On d 21, dietary treatments had no effect on the number of excreta TAB and *Clostridium* spp., whereas, the birds fed the PC and 60 mg AMP-P5/kg diets had fewer (P<0.05; Table 4) excreta coliforms than birds fed the NC and 40 mg AMP-P5/kg diets. Increasing levels of the AMP-P5 in the diets (linear, P<0.05) reduced the number of excreta coliforms. On d 35, birds fed the PC and 60 mg AMP-P5/kg diets had fewer (P<0.05) number of the excreta TAB and coliforms than the birds fed the NC diet. Moreover, on d 35, the excreta TAB and coliform population were linearly (P<0.05) reduced with increase in dietary AMP-P5.

On d 35, the numbers of TAB and *Clostridium* spp. remained unaffected in the ileal and cecal digesta of the broilers (Table 4). However, number of the coliforms was reduced (P<0.05) in the AMP-P5-supplemented birds compared with the

Table 2
Effect of dietary supplementation of antimicrobial peptide-P5 (AMP-P5) and antibiotics on the growth performance of broilers.[a,b]

Item	PC	AMP-P5			SEM	P-value[c]		
		0 (NC)	40	60		*T*	*L*	*Q*
Starter (d 0–21)								
Body weight gain (g)	813^{x}	760^{z}	777^{yz}	793^{xy}	9.10	0.003	0.001	0.963
Feed intake (g)	1196	1175	1187	1191	8.21	0.345	0.083	0.538
FCR	1.47^{y}	1.55^{x}	1.53^{xy}	1.50^{xy}	0.01	0.035	0.024	0.789
Finisher (d 22–35)								
Body weight gain (g)	1228^{x}	1111^{y}	1162^{xy}	1207^{x}	13.86	0.002	0.005	0.895
Feed intake (g)	2266	2146	2188	2218	25.71	0.439	0.350	0.927
FCR	1.85	1.93	1.88	1.84	0.02	0.538	0.178	0.964
Overall (d 0–35)								
Body weight gain (g)	1996^{x}	1826^{z}	1893^{y}	1955^{xy}	17.99	0.002	0.001	0.876
Feed intake (g)	3462	3320	3375	3409	26.89	0.322	0.277	0.785
FCR	1.73	1.82	1.78	1.74	0.02	0.216	0.113	0.899

Means within a row without a common superscript are significantly different (P<0.05).

[a] The dietary treatments were: NC: negative control (basal diet without any antimicrobials); PC: positive control (basal diet + 15 mg avilamycin/kg diet) and AMP-P5 (basal diet supplemented with 40 and 60 mg/kg diet AMP-P5). The NC (diet without antimicrobials) was considered as 0 mg/kg diet AMP-P5.

[b] Data are means of 4 pens of 20 birds each.

[c] *T*: overall effect of treatments; *L*: linear effect of increasing AMP-P5; *Q*: Quadratic effect of increasing AMP-P5 (0, 40 and 60 mg/kg diet).

Table 3
Effect of dietary supplementation of antimicrobial peptide-P5 (AMP-P5) and antibiotics on the nutrient retention (%) in broilers.[a,b]

Item	PC	AMP-P5			SEM	P-value[c]		
		0 (NC)	40	60		*T*	*L*	*Q*
Starter (d 20–21)								
Dry matter	80.81^{x}	77.81^{z}	78.73^{yz}	79.80^{xy}	0.57	0.010	0.006	0.884
Nitrogen	71.96^{x}	68.38^{z}	69.27^{yz}	70.42^{xy}	0.61	0.010	0.007	0.803
Finisher (d 34–35)								
Dry matter	78.44^{x}	76.04^{z}	76.75^{yz}	77.97^{xy}	0.62	0.013	0.015	0.654
Nitrogen	69.08^{x}	66.11^{z}	67.06^{yz}	68.73^{xy}	0.71	0.002	0.030	0.541

Means within a row without a common superscript are significantly different (P<0.05).
[a] The dietary treatments were: NC: negative control (basal diet without any antimicrobials); PC: positive control (basal diet + 15 mg avilamycin/kg diet) and AMP-P5 (basal diet supplemented with 40 and 60 mg/kg diet AMP-P5). The NC (diet without antimicrobials) was considered as 0 mg/kg diet AMP-P5.
[b] Data are means of 4 pens of 2 birds each.
[c] *T*: overall effect of treatments; *L*: linear effect of increasing AMP-P5; *Q*: quadratic effect of increasing AMP-P5 (0, 40 and 60 mg/kg diet).

NC group in both segments of the intestine. In addition, population of in the cecal and ileal digesta was linearly (P<0.05) reduced with increase in dietary AMP-P5.

3.4. Intestinal morphology

The villus height (duodenum and jejunum) and VH:CD (duodenum and ileum) were greater (P<0.05; Table 5) in birds fed the PC and 60 mg AMP-P5/kg diets compared with birds fed the NC diet. The villus height (duodenum and jejunum) and VH:CD (duodenum and ileum) of birds fed the 40 mg AMP-P5/kg diet were not different (P>0.05) from birds fed the NC and 60 mg AMP-P5/kg diets. Moreover, the villus height (duodenum and jejunum) and VH:CD (duodenum, jejunum and ileum) were linearly increased (P<0.05) with increase in dietary AMP-P5. The jejunal crypt depth was linearly decreased (P<0.05) with increase in dietary AMP-P5.

4. Discussion

It has been reported that antimicrobial activity of natural AMPs can be improved by designing analog peptides by modifying the structural properties of the natural peptides (Maloy and Kari, 1995; Javadpour et al., 1996). In present study, we used an analog (AMP-P5) of hybrid antimicrobial peptide CA-MA as a dietary supplement for broilers and its effects on the growth performance, nutrient retention, excreta and intestinal digesta microflora and intestinal morphology were evaluated.

In the present experiment, supplementation of the avilamycin and increasing levels of the AMP-P5 to broiler diet improved the growth performance and retention of DM and N. Our results are in agreement with data reported by Ohh et al. (2009)

Table 4
Effect of dietary supplementation of antimicrobial peptide-P5 (AMP-P5) and antibiotics on the excreta (d 21 and 35) and intestinal (d 35) microbial populations (Log_{10} CFU/g) in broilers.[a,b]

Item	PC	AMP-P5			SEM	P-value[c]		
		0 (NC)	40	60		*T*	*L*	*Q*
d 21								
TAB[d]	8.49	8.74	8.61	8.54	0.04	0.139	0.097	0.786
Clostridium spp.	7.29	7.49	7.41	7.39	0.03	0.343	0.367	0.785
Coliforms	6.55^{y}	6.87^{x}	6.79^{x}	6.61^{y}	0.04	0.004	0.001	0.212
d 35								
TAB	8.38^{y}	8.70^{x}	8.53^{xy}	8.46^{y}	0.06	0.029	0.030	0.541
Clostridium spp.	7.21	7.44	7.35	7.25	0.05	0.098	0.070	0.927
Coliforms	6.37^{y}	6.81^{x}	6.62^{xy}	6.47^{y}	0.08	<0.001	0.001	0.800
Ileum								
TAB	8.23	8.53	8.48	8.30	0.05	0.305	0.225	0.676
Clostridium spp.	6.96	7.15	7.10	7.03	0.04	0.202	0.163	0.895
Coliforms	4.25^{z}	4.69^{x}	4.42^{y}	4.34^{yz}	0.05	0.001	0.003	0.261
Cecum								
TAB	8.35	8.64	8.49	8.39	0.06	0.138	0.069	0.803
Clostridium spp.	7.03	7.23	7.12	7.09	0.04	0.330	0.088	0.525
Coliforms	4.29^{z}	4.78^{x}	4.49^{y}	4.42^{yz}	0.05	0.001	0.001	0.120

Means within a row without a common superscript are significantly different (P<0.05).
[a] The dietary treatments were: NC: negative control (basal diet without any antimicrobials); PC: positive control (basal diet + 15 mg avilamycin/kg diet) and AMP-P5 (basal diet supplemented with 40 and 60 mg/kg diet AMP-P5). The NC (diet without antimicrobials) was considered as 0 mg/kg diet AMP-P5.
[b] Data are means of 4 pens of 2 birds each.
[c] *T*: overall effect of treatments; *L*: linear effect of increasing AMP-P5; *Q*: quadratic effect of increasing AMP-P5 (0, 40 and 60 mg/kg diet).
[d] TAB: total anaerobic bacteria.

Table 5
Effect of dietary supplementation of antimicrobial peptide-P5 (AMP-P5) and antibiotics on the small intestinal morphology in broiler (d 35).[a,b]

Item	PC	AMP-P5			SEM	P-value[c]		
		0 (NC)	40	60		*T*	*L*	*Q*
Villus height, μm								
Duodenum	1933^{x}	1746^{y}	1863^{xy}	1897^{x}	23.81	0.014	0.010	0.323
Jejunum	1164^{x}	1060^{y}	1120^{xy}	1185^{x}	18.22	0.019	0.021	0.945
Ileum	584	515	547	572	11.57	0.142	0.102	0.968
Crypt depth, μm								
Duodenum	632	661	639	628	10.20	0.359	0.232	0.786
Jejunum	414	447	434	412	6.50	0.131	0.046	0.762
Ileum	225	249	234	214	8.08	0.219	0.202	0.912
VH/CD[d]								
Duodenum	3.07^{x}	2.65^{y}	2.80^{xy}	3.02^{x}	0.05	0.023	0.012	0.404
Jejunum	2.71	2.38	2.58	2.88	0.07	0.086	0.010	0.724
Ileum	2.62^{x}	2.10^{y}	2.35^{xy}	2.69^{x}	0.09	0.043	0.017	0.899

Means within a row without a common superscript are significantly different (P<0.05).

[a] The dietary treatments were: NC: negative control (basal diet without any antimicrobials); PC: positive control (basal diet + 15 mg avilamycin/kg diet) and AMP-P5 (basal diet supplemented with 40 and 60 mg/kg diet AMP-P5). The NC (diet without antimicrobials) was considered as 0 mg/kg diet AMP-P5.

[b] Data are means of 4 pens of 2 birds each.

[c] *T*: overall effect of treatments; *L*: linear effect of increasing AMP-P5; *Q*: quadratic effect of increasing AMP-P5 (0, 40 and 60 mg/kg diet).

[d] VH/CD: villus height to crypt depth ratio.

who observed linear improvement in the overall weight gain and retention of DM (starter phase) and CP (starter and finisher phase) in broilers fed diets supplemented with increasing level of AMPs isolated from *Solanum tuberosum*. Similar to the present findings, it was reported that broilers fed diets supplemented with AMPs isolated from the swine gut had greater weight gain and FCR compared with birds fed a non-supplemented diet (Bao et al., 2009; Wang et al., 2009). Contrary to the present results, Ohh et al. (2010) reported that there is no effect of dietary supplementation of the potato peptide on the growth performance of broilers. This discrepancy in results might be due to variation in the type of AMPs used, the level of dietary supplementation, or the mode of action of the AMPs. In the present study, improved growth performance in broilers fed the avilamycin and AMP-P5 might be due to greater nutrient retention, improved intestinal morphology and reduced intestinal coliform populations. The growth performance and the retention of DM and N in birds fed diet supplemented with avilamycin and 60 mg/kg diet AMP-P5 are comparable, which indicates the potential of the AMP-P5 as an antimicrobial growth promoter in broiler diets.

In this experiment, supplementation with AMP-P5 and avilamycin to broiler diets had potential for reducing harmful microflora like excreta and intestinal (ileal and cecal) digesta coliforms. Previous studies showed that dietary supplementation of AMPs isolated from *S. tuberosum* has reduced excreta and cecal digesta coliform count in broilers (Ohh et al., 2009; Ohh et al., 2010). In consistency with present results, Ohh et al. (2010) reported that birds fed diets containing antibiotics had lesser coliforms in excreta and cecal digesta. The AMPs beneficially affect the host animal by improving its intestinal balance and creating gut micro-ecological conditions that suppress harmful microorganisms like coliforms and by favoring beneficial microorganisms like *Lactobacillus* and *Bifidobacterium* (Wang et al., 2007; Jin et al., 2008; Tang et al., 2009; Ohh et al., 2010). Our results suggest that the AMP-P5 has potential for suppressing harmful intestinal microflora in broilers and can be used as a potential antimicrobial growth promoter.

In the present study, supplementation of broiler diet with avilamycin and increasing level of the AMP-P5 increased the villus height of the duodenum and jejunum and VH:CD of the duodenum, jejunum and ileum, suggesting an increased epithelial cell turnover. In line with 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 the AMP isolated from pig small intestine. Similarly, it was reported that birds fed diet supplemented with the rabbit sacculus rotundus AMP had greater villus height of the duodenum and jejunum, but no effect on the ileum (Liu et al., 2008). Increased villus height and VH:CD of the jejunum and ileum were also reported in weanling pigs fed diet supplemented with the expressed fusion peptide bovine lactoferricin–lactoferrampin (Tang et al., 2009). Increasing the villus height suggests an increased surface area capable of greater absorption of available nutrients (Caspary, 1992). Increased villus height and VH:CD 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 experiment, growth performance, nutrient retention, excreta and intestinal microflora populations and intestinal morphology were comparable among birds fed diets supplemented with antibiotic and 60 mg/kg diet AMP-P5. Our results indicate that 60 mg/kg AMP-P5 can be used as novel alternative to antibiotics growth promoter.

5. Conclusion

The results obtained in present study indicate that the dietary supplementation with AMP-P5 has a potential to improve the growth performance, nutrient retention, intestinal morphology and reduce pathogenic bacteria in excreta and intestine

of broilers. However, further studies are required to identify the exact mechanism of action of AMP-P5 that underlies these observations.

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