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5

6 Abstract

7 This study measured the potential changes of the microbiota in the gastrointestinal tract and energy and
8 nutrient digestibility by supplemental bacteriophages in pigs. Twelve castrated male pigs (initial mean
9 body weight = 29.5 ± 2.3 kg) were surgically cannulated using T-cannula. The animals were housed
10 individually in pens equipped with a feeder and a nipple waterer. The pigs were allotted to 1 of 3
11 experimental diets in a quadruplicated 3×2 Latin square design with 3 experimental diets, 2 periods,
12 and 12 pigs resulting in 8 replicates per diet. The 3 diets were a control mainly based on corn and
13 soybean meal with no antibiotics or bacteriophages, a diet containing 0.1% antibiotics, and a diet
14 containing 0.2% bacteriophages. On day 5 of the experimental period, feces were collected and on days
15 6 and 7, ileal digesta were collected. Genomic DNA for bacteria were extracted from the ileal digesta
16 and feces and the V4 region of the 16S rRNA gene was amplified. The ileal and fecal digestibility of
17 energy, dry matter, organic matter, crude protein, and fiber was unaffected by dietary antibiotics or
18 bacteriophages. At the phylum level, the supplemental antibiotic or bacteriophage tended to result in a
19 higher proportion of *Firmicutes* ($p = 0.059$) and a lower proportion of *Bacteroidetes* ($p = 0.099$) in the
20 ileal digesta samples compared with the control group with no difference between the antibiotic and
21 bacteriophage groups. At the genus level, the supplemental antibiotic or bacteriophage tended to result
22 in a higher proportion of *Lactobacillus* ($p = 0.062$) and a lower proportion of *Bacteroides* ($p = 0.074$)
23 and *Streptococcus* ($p = 0.088$) in the ileal digesta compared with the control group with no difference
24 between the antibiotic and bacteriophage groups. In the feces, supplemental antibiotics or
25 bacteriophages reduced the proportion of *Bifidobacterium* compared with the control group ($p = 0.029$)
26 with no difference between the antibiotic and bacteriophage groups. Overall, supplemental antibiotics
27 and bacteriophages showed positive effect on the microbiota of in the ileal digesta without largely
28 affecting energy or nutrient digestibility, with no differences between the antibiotic and bacteriophage
29 groups in growing pigs.

30

31 **Keywords:** Additive, Antibiotic, Digestibility, Probiotic, Pig, *Lactobacillus*

32

33 **Introduction**

34 For several decades, antibiotics have been used in pig diets to promote growth, improve feed
35 efficiency, and prevent diseases [1]. However, the use of antibiotics is a concern because of the potential
36 occurrence of antibiotic-resistant bacteria and residual antibiotics in pork [2, 3]. Hence, the swine feed
37 industry in many countries has prohibited the use of antibiotics in swine diets, and identifying
38 alternatives has become a research focus [4-7]. Bacteriophages (BP) have received attention as
39 appropriate antibiotic alternatives and many studies on BP have been conducted in pigs [8-10].

40 As the BP are non-hazardous and self-replicating agents that are parasitic on bacteria as host,
41 they have been suggested to infect and grow on bacteria [11]. Therefore, supplemental BP in pig diets
42 may mimic the antimicrobial effects of antibiotics, promoting the digestion and absorption of nutrients
43 by eliminating detrimental bacteria in the intestinal tract. Previous studies reported that supplemental
44 BP in pig diets increases the apparent total tract digestibility (ATTD) of energy and nutrients [12] and
45 decreases the concentration of *Escherichia coli* and *Salmonella* in feces [13]. In addition, a recent study
46 reported that supplemental BP improved intestinal barrier function and intestinal microbiota in nursery
47 pigs [10]. However, information on the influence of BP on changes of microbial concentrations in the
48 small intestine and ileal nutrient digestibility is lacking. Thus, to bridge this gap, the present work aimed
49 to determine the effects of BP on the relative proportions of microbiota in the ileal digesta and feces
50 and on nutrient digestibility in pigs.

52 **Materials and Methods**

53 **Ethical declaration**

54 The experimental protocols were reviewed and approved by the Institutional Animal Care and
55 Use Committee of Konkuk University (KU15016).

57 **Animals, diets, and experimental design**

58 Twelve crossbred castrated male pigs with an initial mean body weight of 29.5 kg (standard
59 deviation = 2.3) were employed to assess the effect of BP on microbial concentrations in the small and
60 large intestines, as well as energy and nutrient digestibility. Pigs were fitted with a T-cannula at the
61 distal ileum using the surgical procedure described by Stein et al. [14]. The animals were individually
62 housed in pens (2.0 × 2.2 m) equipped with a feeder and a nipper waterer. The 12 animals were randomly
63 allotted to one of 3 dietary treatment groups in a quadruplicated 3 × 2 Latin square design, with 3
64 experimental diets and 2 periods per square. Potential residual effects were balanced using a spreadsheet
65 program developed by Kim and Kim [15].

66 The 3 experimental diets were (Table 1): a control diet mainly based on corn and soybean meal,
67 with 0.2% ground corncob and wheat shorts as BP carriers; an antibiotic diet (AB), supplemented with
68 0.1% antibiotics (avilamycin; Avilamix, CTCBIO Inc., Seoul, Korea); and a BP diet supplemented with

69 0.2% BP at the expense of the BP carriers in the control diet. The energy and nutrient concentrations in
70 all diets met or exceeded the requirement estimates suggested by the NRC [16]. All experimental diets
71 contained chromic oxide at 0.5% as an indigestible index. The BP product was obtained from CTCBIO
72 Inc. (Seoul, Republic of Korea). The product contained a mixture of BP consisted of *Clostridium*
73 *perfringens* types A and C, *Escherichia coli* (f41, k88, and k99), *Salmonella* spp. (*Salmonella*
74 *choleraesuis*, *Salmonella derby*, *Salmonella enteritidis*, and *Salmonella typhimurium*), and
75 *Staphylococcus aureus*. The BP product contained 10^9 plaque-forming units per gram.

76

77 **Feeding and sample collection**

78 The animals were fed the experimental diets at 3 times the daily maintenance requirement for
79 energy (i.e., 197 kcal metabolizable energy/kg BW^{0.60}; [16]). The amount of feed allowance per pig was
80 divided into 2 equal meals and provided to pigs at 0800 and 1700 h. Water was freely available. An
81 experimental period was consisted of 4 days of adaptation to the experimental diet, 1 day of fecal
82 collection, and 2 days of ileal digesta collection. Fecal samples were collected from 0900 to 1800 on
83 day 5 by grab sampling, and rectal massage was carried out to acquire fresh fecal samples for microbial
84 community analysis. Ileal digesta samples were collected from 0900 to 1630 on days 6 and 7 using a
85 wired plastic bag fixed to a T-cannula. The plastic bags were replaced whenever filled with ileal digesta,
86 or at least every 30 min. Collected feces and ileal digesta were immediately stored at -20°C for further
87 analyses. After the termination of the first period, 4 days of buffering period was used to prevent
88 potential carryover effects.

89

90 **Microbial community and chemical analyses**

91 Bacterial genomic DNA were extracted from fresh ileal and fecal samples and the V4 region of
92 the 16S rRNA gene was amplified. Amplicons were sequenced on an Illumina MiSeq, and microbial
93 communities and alpha diversity were analyzed using Quantitative Insights into Microbial Ecology as
94 described by Han et al. [17].

95 Fecal samples were dried in a forced-air drying oven and ileal digesta samples were lyophilized
96 in a freeze dryer and finely ground (< 1 mm) before chemical analysis. Diet, feces, and ileal digesta
97 samples were analyzed for dry matter (AOAC [18]; method 930.15), crude protein (AOAC [18]; method
98 990.03), and chromium (AOAC [18]; method 990.08) contents. The fibers were analyzed (D200;
99 Ankom Technology, Macedon, NY, USA) for neutral detergent fiber (Ankom Technology, 1998a;
100 method 13) and acid detergent fiber (Ankom Technology, 1998b; method 12) contents. All samples and
101 diets were analyzed for gross energy using bomb calorimetry (Model C2000, IKA®, Germany).

102

103 **Calculations and statistical analyses**

104 The apparent ileal digestibility (AID) and ATTD of energy and nutrients were calculated using
105 the index method [19]. Experimental data were analyzed using the MIXED procedure of SAS 9.4 (SAS

106 Institute. Inc., Cary, NC, USA). The statistical model included dietary treatment as the fixed variable
107 and replication, period within replication, and animal within replication as random variables. The
108 proportion of microbial communities in each treatment was expressed as a percentage of the total 16S
109 rRNA gene sequences. Least squares means for the response variables were calculated for each
110 treatment. Orthogonal contrasts were used to compare the control versus the AB and BP groups and the
111 AB group versus the BP group. An individual pig was the experimental unit. Statistical significance and
112 trend were determined at $p \leq 0.05$ and $0.05 < p \leq 0.10$, respectively.

113

114 **Results**

115 One pig that was provided with the control diet declined to consume the diet during the
116 collection period and was subsequently excluded from the data for statistical analysis.

117

118 **Microbial communities at different gut locations**

119 A total of 528,719 (mean = $11,494 \pm 575$) 16S rRNA reads were generated, with an average of
120 12,361 ($\pm 1,465$) and 9,970 ($\pm 1,627$) reads in ileal digesta and feces from the pigs fed the control diet,
121 12,787 (± 699) and 10,633 ($\pm 1,627$) reads in ileal digesta and feces from the pigs fed the AB diet and
122 12,932 ($\pm 1,761$) and 10,198 ($\pm 1,059$) reads in ileal digesta and feces from the pigs fed the BP diet,
123 respectively.

124 The alpha diversity of the microbial communities in the ileum and feces was not affected by
125 AB or BP supplementation (Fig. 1 and 2). At the phylum level, *Firmicutes* accounted for the largest
126 proportion of the microbiota in the ileal and fecal samples of all groups, followed by *Bacteroidetes*
127 (Table 2). The proportion of *Bacteroidetes* in the ileal digesta tended to be less ($p = 0.099$) in the pigs
128 fed the AB- or BP-supplemented diets compared with the control group, but there was no difference
129 between AB and BP groups. The proportion of *Firmicutes* in the ileal digesta tended to be greater ($p =$
130 0.059) in the pigs fed the AB or BP diets than in the control group but there was no difference between
131 the AB and BP groups. In the feces, there were no differences in the relative proportions of
132 *Bacteroidetes* and *Firmicutes* among all treatments.

133 At the genus level, the proportion of *Lactobacillus* in the ileal digesta was the most dominant
134 and tended to be greater ($p = 0.062$) in the pigs fed the AB- or BP-supplemented diets than in the control
135 group, with no difference between the AB and BP groups (Table 3). The proportions of *Bacteroides* (p
136 $= 0.074$) and *Streptococcus* ($p = 0.088$) in the ileal digesta tended to be less in the AB and BP groups
137 compared to the control group with no difference between the AB and BP groups. In the fecal samples,
138 the relative proportion of *Lactobacillus* was the most dominant. The relative proportion of
139 *Bifidobacterium* tended to be less ($p = 0.029$) in the AB and BP groups compared with the control group
140 with no difference between the AB and BP groups. In contrast, the relative proportions of
141 *Parabacteroides* and *Succinivibrio* were less ($p < 0.05$) in the AB group compared with the BP group.

142

143 **Energy and nutrient digestibility at different gut locations**

144 The AID and ATTD of energy and nutrients were not affected by the supplemental AB or BP
145 (Table 4).

146

147 **Discussion**

148 The BP bind to specific receptors on the surface of bacteria prior to introducing their genetic
149 materials [20, 21]. The relationship between BP and bacteria is either lytic or lysogenic. During lytic
150 infection, the phages adhere to the surface of bacteria and they inject their chromosomes into the
151 bacterial cells. After that, the phages reproduce and release virulent phages. In the lysogenic cycle, the
152 genetic materials of phages incorporate into bacterial chromosomes. This incorporation permits bacteria
153 to reproduce generally along with phage genetic material known as prophages. Consequently, temperate
154 phages are released, and these have the potential to convert into virulent phages at any time [22]. Thus,
155 the antimicrobial potential of virulent phages is greater than that of temperate phages. Each type of BP
156 can efficiently infect specific bacteria more efficiently than others [23]. As the BP product used in this
157 study contained a cocktail of BP for *Clostridium perfringens* types A and C, *Escherichia coli*
158 *Salmonella* spp., and *Staphylococcus aureus*, the phage product may be specifically effective against
159 these bacteria. Kim et al. [24] reported that supplemental BP at 0.1% or 0.15% decreased ileal
160 *Escherichia coli* and *Clostridium* proportion in pigs. Furthermore, a previous study found that
161 supplemental BP against *Salmonella typhimurium* increased the body weight gain and feed efficiency
162 in *Salmonella*-challenged growing pigs [25].

163 Avilamix containing 20 g/kg avilamycin was used in the present study. Avilamycin is an
164 antibiotic that inhibits protein synthesis by selectively targeting bacterial ribosomes. This action
165 disrupts protein production, effectively suppressing bacterial growth and demonstrating antibacterial
166 activity [26]. A previous study observed a significant reduction in *Salmonella* count in chicks treated
167 with 100 ppm avilamycin compared to the control group [27].

168 The overall microbial diversity comprised prevalent intestinal microbial groups, with
169 *Firmicutes* (35%), *Bacteroidetes* (21%), *Proteobacteria* (3%), and *Spirochaetes* (2%) dominating the
170 total 16S rRNA gene sequences, as previously observed in pigs [28, 29]. However, according to a meta-
171 analysis to define the core microbiota in the gastrointestinal tract of pigs, the ileal digesta primarily
172 consisted of *Firmicutes* and *Proteobacteria*, whereas the phylum composition in the cecum and colon
173 exhibited a high level of consistency [30]. In the ileal digesta in the present study, *Bacteroidetes* were
174 10 percentage unit greater than that reported in the literature. This inconsistency might be explained by
175 different ileal digesta sampling methods. Most studies in the meta-analysis obtained ileal samples by
176 necropsy at the end of the experiment [31-33]. In contrast, the collection of ileal digesta in this study
177 used a T-cannula inserted into the terminal ileum, approximately 15 cm from the ileocecal valve [14].

178 Because the flow of ileal digesta in the small intestine is not consistent in one direction, the ileal digesta
179 collected using a T-cannula may contain some cecal digesta. The analytical procedure, such as the
180 targeted region of the 16S rRNA gene and DNA purification methods, also affects the composition of
181 the microbial community [34].

182 Intestinal microbiota plays a critical role in maintaining immune function, nutritional status,
183 and physiology in pigs. [35, 36]. Accordingly, major or frequent changes in intestinal microbiota are
184 often associated with ill health [37-39]. *Firmicutes* and *Bacteroidetes* are the 2 dominant phyla in the
185 intestine of pigs [40]. The proportions of *Firmicutes* and *Bacteroidetes* in the intestine are associated
186 with the maintenance of homeostasis and changes in their proportions can lead to various pathologies.
187 It has been reported that a decrease in the proportion of *Bacteroidetes* together with an increase in the
188 proportion of *Firmicutes* can create an intestinal environment conducive to energy production and
189 absorption in the intestine [41, 42]. To our knowledge, we first report the effects of BP on microbiota
190 at the phylum level in the ileal digesta of pigs. The effects of BP on the alteration of microbiota at the
191 phylum level in the ileal digesta were similar to those of AB. However, the effects of AB or BP on fecal
192 microbiota at the phylum level have shown inconsistent results compared to the ileal digesta, which
193 may be explained by the experimental period. Fecal samples were collected after 4 days of the
194 adaptation period, which could be insufficient to detect noticeable changes. However, ileal digesta
195 samples were collected after 5 days of adaptation and the fecal collection period, which might be
196 sufficient to detect the effects of supplemental AB or BP.

197 *Bifidobacterium* and *Lactobacillus* exert beneficial effects on the intestinal environment of
198 animals [43-45]. The growth of these beneficial bacteria was enhanced by the addition of AB. Because
199 the antimicrobial action of AB reduces the colonization of harmful bacteria, it is likely to result in an
200 increase in probiotics in a less competitive intestinal environment [46]. The avilamycin used in this
201 study is recognized for its primary targeting of *Staphylococcus aureus*, *Streptococcus pneumoniae*, and
202 *Streptococcus pyogenes* [26]. However, our study did not detect these specific harmful bacteria and
203 previous research indicated no significant impact on the normal gut flora [47]. This aligns with our
204 findings, suggesting that microbial diversity remains unaffected by avilamycin administration. In this
205 study, we hypothesized that microbes affected by avilamycin could be found among the low-abundance
206 microbes.

207 The increased proportion of *Lactobacillus* in the ileum with supplemental AB or BP was likely
208 due to the inhibitory action of AP or BP on pathogenic bacteria. To our knowledge, only one study
209 determined the effect of BP on the microbiota of this genus in the ileal digesta of pigs. Kim et al. [24]
210 reported that supplemental BP at 0.10% or 0.15% increased the ileal *Lactobacillus* proportion but had
211 no effect on the fecal *Lactobacillus* proportion in pigs, which agrees with the present results. However,
212 previous studies have reported that supplemental BP increased the fecal *Lactobacillus* proportion in
213 pigs [8, 12, 13]. The reason for the inconsistency between the present and previous studies is unclear.

214 The less proportion of *Bifidobacterium* in the feces observed in the AB or BP group compared
215 with the control group was inconsistent with previous studies [8, 12, 24], the reason for which remains
216 unclear. However, another study reported that a supplemental AB mixture consisting of ampicillin,
217 gentamycin, and metronidazole resulted in decreased *Bifidobacterium* abundance in the feces of pigs
218 [48], which agrees with the present study. They observed that the fecal *Bifidobacterium* proportion was
219 positively correlated with branched-chain fatty acid concentration in the feces of pigs fed AB.
220 Branched-chain fatty acids are formed from branched-chain amino acids that originate exclusively from
221 the breakdown of proteins and thus serve as indicators of microbial branched-chain amino acid
222 deamination. Thus, they suggested that the reduction in branched-chain fatty acids due to the effect of
223 AB on microbial nitrogen results in a decreased *Bifidobacterium* proportion in the feces [48].

224 At the genus level in the gut, the target bacteria of BP containing *Clostridium perfringens*,
225 *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus* were not detected in this study. However,
226 the proportion of *Streptococcus* in the ileal digesta decreased with the addition of AB or BP.
227 *Streptococcus* is a genus of bacteria and certain species within this genus are known to cause various
228 diseases. However, the results of this observation are unclear because of the known host specificity of
229 BP.

230 The ATTD of energy and nutrients in the control diet was within the range of previous data [49,
231 50]. The index method used to calculate digestibility in this study relies greatly on the accurate chemical
232 analysis of index compounds in the feed and digesta, which requires sufficient adaptation to achieve a
233 constant fecal index concentration. Choi and Kim. [51] suggested that a minimum adaptation time of
234 3.5 days is needed to achieve a constant fecal index concentration if a high-fiber diet containing 30%
235 neutral detergent fiber is fed, whereas 5.5 days are needed for a low-fiber diet containing 5% neutral
236 detergent fiber. Because the neutral detergent fiber concentration of the experimental diets in this study
237 was greater than 5%, an adaptation period of 4 days was sufficient.

238 The intestinal microbiota helps the host utilize nutrients and defend against pathogens [52].
239 However, in the present study, BP supplementation did not affect nutrient digestibility despite having
240 positive effects on the intestinal microbial community. There are two potential reasons for these results.
241 First, the species of microbes are critical for significant changes in nutrient digestibility. Niu et al. [53]
242 suggested that only specific microbial species improve nutrient digestibility. In their study, the
243 proportions of 3 phyla of *Proteobacteria*, *Tenericutes*, and *TM7*, and 11 genera including *Anaeroplasma*,
244 *Campylobacter*, and *Clostridium* were positively correlated with apparent crude fiber digestibility.
245 However, in the present study, there were no differences in the abundance of these phyla or genera
246 among the treatments. Second, the experimental conditions, such as the use of a variety of AB,
247 experimental diet composition, and experimental period, differed among the studies. There are
248 discrepancies among studies on the relationships between microbial communities and nutrient
249 digestibility in pigs. A strong relationship between the intestinal microbiota and nutrient digestibility in
250 pigs has been reported in multiple studies [12, 13, 48] whereas no relationship was observed in other

251 studies [54, 55]. These discrepancies were attributed to the fact that the magnitude of microbial changes
252 was not large and perhaps would not improve nutrient digestibility because of the short experimental
253 period compared to previous studies. Previous studies fed diets containing 0.05% [13] or 0.1% [12] BP
254 for more than 30 days, whereas this experiment was conducted for only 7 days. In addition, Kim et al.
255 [24] reported that supplementation with BP at 0.15% resulted in increased apparent total tract dry matter
256 digestibility for 35 days, but had no effect when fed for 7 days.

257 In conclusion, although BP supplementation had no effect on energy and nutrient digestibility
258 and seemed to require a relatively long adaptation time to improve digestibility, the alteration of the
259 intestinal microbiota in the ileal digesta and feces in the BP group was similar to that in the AB group.
260 This might be the basis for supplemental BP to show a function similar to that of antibiotics. However,
261 the effects of supplemental BP on the intestinal microbiota were inconsistent with those reported in
262 previous studies. In addition, studies on the effect of BP on ileal microbiota at the phylum level and
263 ileal digestibility in pigs are scarce. Therefore, further studies to determine the effects of BP on the ileal
264 microbiota should be conducted to bridge this gap.

265

266

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440 **Table 1.** Ingredient and analyzed nutrient composition of experimental diets (as-fed basis, %)

Item	Treatment		
	Control	Antibiotic (AB) ¹⁾	Bacteriophage (BP) ²⁾
Ingredient composition			
Corn, yellow dent	64.35	64.35	64.35
Soybean meal, 48% crude protein	30.00	30.00	30.00
Soybean oil	2.00	2.00	2.00
AB	-	0.10	-
BP	-	-	0.20
BP carrier ³⁾	0.20	0.10	-
L-Lys ·HCl, 78.8 %	0.10	0.10	0.10
Dicalcium phosphate	1.10	1.10	1.10
Limestone	0.85	0.85	0.85
Vitamin-mineral premix ⁴⁾	0.50	0.50	0.50
Salt	0.40	0.40	0.40
Chromic oxide	0.50	0.50	0.50
Analyzed nutrient composition			
Dry matter	92.2	92.6	92.6
Organic matter	86.5	87.0	87.1
Crude protein	20.2	20.2	20.2
Neutral detergent fiber	11.9	11.8	11.8
Acid detergent fiber	3.6	3.7	3.6
Gross energy, kcal/kg	4,175	4,198	4,198

441 ¹⁾ Avilamix (CTCBIO Inc., Seoul, Republic of Korea) contained 20 g of avilamycin/kg.

442 ²⁾ BacterPhage (CTCBIO, Inc., Seoul, Republic of Korea) contained contained a mixture of BP consisted
 443 of *Clostridium perfringens* types A and C, *Escherichia coli* (f41, k88, and k99), *Salmonella* spp.
 444 (*Salmonella choleraesuis*, *Salmonella derby*, *Salmonella enteritidis*, and *Salmonella typhimurium*), and
 445 *Staphylococcus aureus*. The BP product contained 10⁹ plaque-forming units per gram.

446 ³⁾ Consisted of ground corn cob and wheat shorts.

447 ⁴⁾ Provided the following quantities per kg of complete diet: vitamin A, 25,000 IU; vitamin D₃, 4,000
 448 IU; vitamin E, 50 IU; vitamin K, 5.0 mg; thiamin, 4.9 mg; riboflavin, 10.0 mg; pyridoxine, 4.9 mg;
 449 vitamin B₁₂, 0.06 mg; pantothenic acid, 37.5 mg; folic acid, 1.10 mg; niacin, 62 mg; biotin, 0.06 mg;
 450 Cu, 25 mg as copper sulfate; Fe, 268 mg as iron sulfate; I, 5.0 mg as potassium iodate; Mn, 125 mg as
 451 manganese sulfate; Se, 0.38 mg as sodium selenite; Zn, 313 mg as zinc oxide; butylatedhydroxytoluene,
 452 50 mg.

Table 2. Effects of supplemental antibiotic (AB) and bacteriophage (BP) on relative proportion of phylum and *Firmicutes*-to-*Bacteroidetes* ratio (F:B) in the ileal and fecal microbiota of growing pigs¹⁾

Item ²⁾	Treatment				<i>p</i> -values	
	Control	AB ³⁾	BP ⁴⁾	SEM ⁵⁾	Control vs. AB and BP	AB vs. BP
Ileum, %						
<i>Actinobacteria</i>	4.5	4.9	10.3	3.1	0.395	0.179
<i>Bacteroidetes</i>	18.4	14.2	11.9	3.0	0.099	0.492
<i>Firmicutes</i>	60.5	68.7	68.8	3.6	0.059	0.987
<i>Proteobacteria</i>	5.9	6.6	3.8	1.8	0.686	0.172
Others ⁶⁾	10.5	6.1	5.1	2.4	0.102	0.741
Feces, %						
<i>Actinobacteria</i>	5.2	2.3	2.6	1.3	0.056	0.867
<i>Bacteroidetes</i>	27.2	26.9	26.1	4.1	0.892	0.895
<i>Firmicutes</i>	56.9	60.0	59.4	3.5	0.525	0.900
<i>Proteobacteria</i>	2.5	2.9	4.2	0.9	0.275	0.226
Others ⁶⁾	7.6	8.4	7.5	0.9	0.796	0.530

¹⁾ Each least squares mean represents 8 observations except the control diet (n = 7).

²⁾ The taxa at the phylum level with more than 3% average proportion in all samples were selected.

³⁾ Avilamix (avilamycin 20 g/kg, CTCBIO Inc., Seoul, Republic of Korea) was supplemented at 0.1%.

⁴⁾ BacterPhage (CTCBIO Inc., Seoul, Republic of Korea) was supplemented at 0.2%. The product contained a mixture of BP consisted of *Clostridium perfringens* types A and C, *Escherichia coli* (f41, k88, and k99), *Salmonella* spp. (*Salmonella choleraesuis*, *Salmonella derby*, *Salmonella enteritidis*, and *Salmonella typhimurium*), and *Staphylococcus aureus*. The BP product contained 10⁹ plaque-forming units per gram.

⁵⁾ SEM, standard error of the means.

⁶⁾ Phylum less than 0.1% of the average in all groups.

Table 3. Effects of supplemental antibiotic (AB) and bacteriophage (BP) on relative proportion of genus in the ileal and fecal microbiota of growing pigs¹⁾

Item ²⁾	Treatment			SEM ⁵⁾	<i>p</i> -values	
	Control	AB ³⁾	BP ⁴⁾		Control vs. AB and BP	AB vs. BP
Ileum, %						
<i>Clostridium</i>	1.7	1.9	1.5	0.5	0.915	0.496
<i>Lactobacillus</i>	28.0	36.6	38.4	4.1	0.062	0.729
<i>Bacillus</i>	2.7	1.2	1.5	0.6	0.105	0.702
<i>Bacteroides</i>	4.6	3.0	2.4	0.8	0.074	0.560
<i>Bifidobacterium</i>	4.0	4.5	9.7	2.9	0.345	0.142
<i>Megasphaera</i>	2.8	4.1	3.7	0.8	0.295	0.751
<i>Mitsuokella</i>	1.9	1.4	1.7	0.6	0.697	0.762
<i>Prevotella</i>	7.0	6.0	4.9	1.3	0.246	0.407
<i>Streptococcus</i>	2.2	1.4	1.6	0.3	0.088	0.702
<i>Veillonella</i>	0.4	1.2	1.5	0.7	0.277	0.782
Others ⁶⁾	44.4	38.7	33.1	4.0	0.110	0.320
Feces, %						
<i>Clostridium</i>	0.6	0.6	0.6	0.1	0.954	0.734
<i>Lactobacillus</i>	20.2	20.5	20.6	3.7	0.933	0.988
<i>Bacteroides</i>	3.9	4.9	4.0	1.1	0.688	0.484
<i>Bifidobacterium</i>	3.5	1.6	2.0	0.8	0.029	0.589
<i>Faecalibacterium</i>	1.5	1.3	1.2	0.2	0.262	0.737
<i>Megasphaera</i>	3.3	3.4	7.6	2.2	0.386	0.142
<i>Oscillospira</i>	1.4	1.5	1.2	0.2	0.723	0.107
<i>Parabacteroides</i>	1.0	1.4	5.5	1.6	0.245	0.080
<i>Prevotella</i>	7.0	6.5	6.5	1.4	0.801	0.997
<i>Streptococcus</i>	3.4	0.7	0.6	1.4	0.152	0.985
<i>Ruminococcus</i>	1.4	2.4	1.6	0.5	0.344	0.293
<i>Succinivibrio</i>	0.8	0.7	2.3	0.8	0.417	0.090
<i>Treponema</i>	1.4	0.8	1.6	0.5	0.830	0.215
Others ⁶⁾	50.1	54.0	44.4	3.1	0.784	0.032

¹⁾ Each least squares mean represents 8 observations except the control diet (n = 7).

²⁾ The taxa at the genus level with more than 1% average proportion in all samples were selected.

³⁾ Avilamix (avilamycin 20 g/kg, CTCBIO Inc., Seoul, Republic of Korea) was supplemented at 0.1%.

⁴⁾ BacterPhage (CTCBIO Inc., Seoul, Republic of Korea) was supplemented at 0.2%. The product contained a mixture of BP consisted of *Clostridium perfringens* types A and C, *Escherichia coli* (f41, k88, and k99), *Salmonella* spp. (*Salmonella choleraesuis*, *Salmonella derby*, *Salmonella enteritidis*, and *Salmonella typhimurium*), and *Staphylococcus aureus*. The BP product contained 10⁹ plaque-forming units per gram.

⁵⁾ SEM, standard error of the means.

⁶⁾ Genus less than 0.1% of the average in all groups and the unclassified genera.

Table 4. Effects of supplemental antibiotic (AB) and bacteriophage (BP) on apparent ileal and total tract digestibility of energy and nutrients in growing pigs¹⁾

Item	Diet			SEM ⁴⁾	<i>p</i> -values	
	Control	AB ²⁾	BP ³⁾		Control vs. AB and BP	AB vs. BP
Ileal digestibility, %						
Energy	80.7	79.1	79.5	1.4	0.347	0.818
Dry matter	79.6	78.2	78.5	1.5	0.413	0.845
Organic matter	81.5	79.9	80.3	1.6	0.426	0.809
Crude protein	83.8	82.4	81.0	1.4	0.109	0.276
Neutral detergent fiber	45.8	42.1	41.7	3.7	0.411	0.929
Acid detergent fiber	26.9	28.4	30.2	3.9	0.570	0.708
Total tract digestibility, %						
Energy	81.4	82.5	82.4	0.9	0.206	0.917
Dry matter	82.0	82.9	83.0	0.7	0.199	0.966
Organic matter	84.1	84.7	85.0	0.7	0.376	0.752
Crude protein	80.7	82.5	81.9	1.2	0.242	0.626
Neutral detergent fiber	49.8	49.6	52.8	2.5	0.403	0.100
Acid detergent fiber	39.1	37.7	39.9	3.9	0.886	0.380

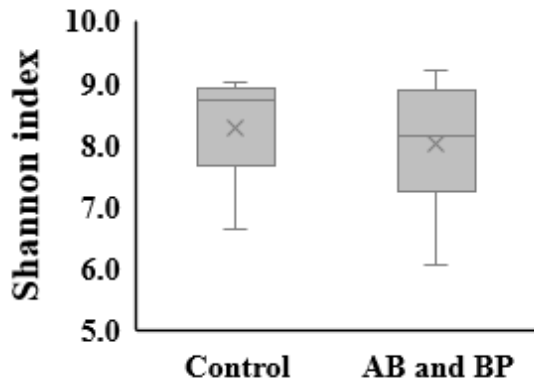
¹⁾ Each least squares mean represents 8 observations except the control diet (n = 7).

²⁾ Avilamix (avilamycin 20 g/kg, CTCBIO Inc., Seoul, Republic of Korea) was supplemented at 0.1%.

³⁾ BacterPhage (CTCBIO Inc., Seoul, Republic of Korea) was supplemented at 0.2%. The product contained a mixture of BP consisted of *Clostridium perfringens* types A and C, *Escherichia coli* (f41, k88, and k99), *Salmonella* spp. (*Salmonella choleraesuis*, *Salmonella derby*, *Salmonella enteritidis*, and *Salmonella typhimurium*), and *Staphylococcus aureus*. The BP product contained 10⁹ plaque-forming units per gram.

⁴⁾ SEM, standard error of the means.

$p = 0.357$
SEM = 0.33



$p = 0.479$
SEM = 0.33

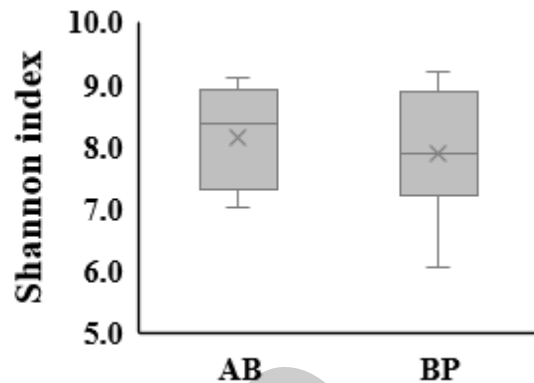
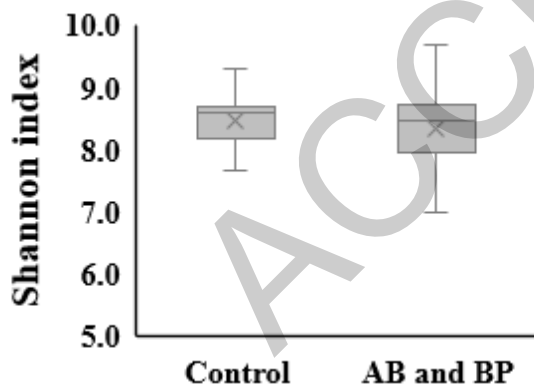


Fig. 1. The alpha diversity of Shannon index for ileum microbial community in pigs fed control, antibiotic (AB), or bacteriophage (BP) diet. SEM, standard error of the means. The X symbols represent the mean values.

$p = 0.650$
SEM = 0.25



$p = 0.426$
SEM = 0.25

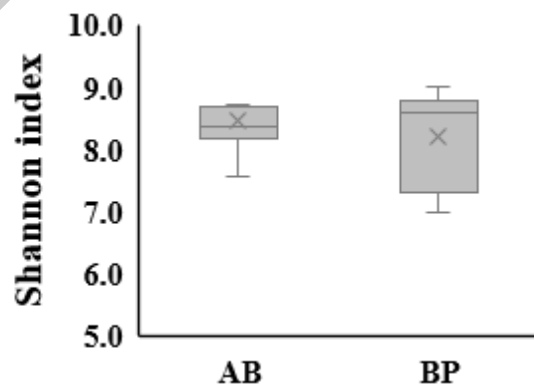


Fig. 2. The alpha diversity of Shannon index for fecal microbial community in pigs fed control, antibiotic (AB), or bacteriophage (BP) diet. SEM, standard error of the means. The X symbols represent the mean values.