Research Article

Effect of Bacteriophages on Intestinal Colonization of *Escherichia coli*, Cecal Microbiota Composition, Intestinal Morphology, and Growth Performance in Nursery Pigs from Commercial Pig Farms

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Abstract | This study was conducted to determine the effects of a bacteriophage cocktail on growth performance, intestinal morphology, *E coli* detection, cecal bacterial composition, and the incidence of diarrhea in nursery pigs for 6 weeks. A total of 800 pigs (Large White × Landrace × Duroc) were randomly allocated to two treatments: a basal diet supplemented with a mixture of amoxicillin and colistin (Amox-Co) and a basal diet supplemented with amoxicillin and 1 g/kg of a bacteriophage cocktail (Amox-Phage). Each treatment consisted of eight replicate pens, with 50 pigs per replicate. Average daily gain (ADG) and the FCR did not differ between the groups. The Amox-Phage group showed greater resistance to diarrhoea compared with the Amox-Co group during week 6 ($p < 0.05$). The Amox-Co group showed greater ($p < 0.05$) villus height at the jejunum and ileum compared with the Amox-Phage group and deeper crypts in the jejunum. Moreover, the goblet cell density in the duodenum was greater in the Amox-Phage group. The total intestinal population of *E. coli* did not differ between the groups ($p > 0.05$), ETEC (F18) and EHEC were not detected. Amox-Phage supplementation did not affect cecal bacterial diversity. Firmicutes was the core phylum in the gut microbiota of nursery pigs, and there was a significantly increased relative abundance of Proteobacteria in the Amox-Phage group. Concurrently, the Amox-Phage group showed an increase in the relative abundance of Lactobacillaceae in the caecum. The results of this study indicate that the bacteriophage cocktail has the potential to alter the abundance of intestinal microbiota without increasing the incidence of diarrhea or negatively affecting growth. Hence, a bacteriophage cocktail could be added to the feed of nursery pigs.

Keywords | Antibiotics, Bacteriophage, *E. coli*, Microbiota, Nursery pigs

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INTRODUCTION

Gut infection of nursery pigs caused by multidrug-resistant (MDR) pathogenic bacteria immediately after weaning is closely associated with farm and feed management and the available health care system (Bruun et al., 2009; Heo et al., 2013). The infection also imposes a significant decrease in nursery pig growth performance and mortality (Hermann-Bank et al., 2015). Enterotoxigenic *E. coli* (ETEC) infection in piglets results in growth re-

tardation, diarrhea, and mortality (Bogere et al., 2019). In swine, ETEC F4 and F18 produce enterotoxins that cause diarrheal diseases (Fairbrother et al., 2005, Nagy et al., 2005, Yang et al., 2017).

In general, the disease is commonly prevented or alleviated by adding antibiotics to the feed of weaned piglets for 3–6 weeks, but the use of antibiotics as a feed additive has been banned in the European Union (EU) since 2006. Thailand's National strategic plan on antimicrobial resistance 2017–2021 was proposed and subsequently endorsed by the Cabinet Resolution on 17 August 2016. Therefore, the Ministry of Agriculture and Cooperatives is responsible for (1) the control and prevention of antimicrobial resistance (AMR) genes in agriculture and animals and (2) the regulation of antimicrobial distribution. In a recent systematic review, Lekagul et al. (2021) reported that in the pig sector of Thailand, antibiotics are commonly used during the suckling and post-weaning stages of production under the regulation of antimicrobial distribution. In addition, antibiotics have led to adverse effects on the beneficial microbiota and the emergence of AMR genes, which is a major threat to human health through drug resistance gene transfer (Ghaisas et al., 2016). Correa-Fiz et al. (2019) reported that antimicrobial treatments change the gut microbiota structure and reduce the health and productivity of piglets. Hence, reducing antibiotic use in commercial pig farms may potentially enhance swine health.

Bacteriophages are proposed as a way to control pathogenic bacteria in the swine farming industry (Kim et al., 2017; Oh et al., 2022). Several studies have shown that bacteriophage therapy can significantly reduce the *E. coli* and *Salmonella* spp. counts in the gastrointestinal tract of pigs (Kim et al., 2014; Seo et al., 2018). Lee et al. (2016) found that dietary supplementation of bacteriophages to nursery pigs with post-weaning diarrhea (PWD) caused by ETEC (F4) reduced *E. coli* adhesion in the ileum and cecum. The effect of bacteriophage dietary supplementation on growth performance has been studied; the inclusion of apramycin and anti-*Salmonella* bacteriophages did not affect the growth performance of growing pigs compared with the basal diet (Yan et al., 2012). The application of a bacteriophage cocktail can inhibit pathogen growth, and weaned pigs fed a bacteriophage cocktail diet had an increased ($p < 0.05$) average daily gain (ADG), gain to feed (G: F) ratio, and fecal score (Kim et al., 2017).

Given the lack of studies investigating the effects of bacteriophages as potential substitutes for antibiotic use in piglets on pig farms, we aimed to study the existing situation in commercial pig farms in Thailand. Moreover, we aimed to identify combinations of dietary supplementation with bacteriophages and amoxycillin compared with the use of in-feed colistin and amoxycillin in nursery pigs. We analyzed the diarrhea score and *E. coli* intestinal colonization to determine the effect on the cecal microbiota composition and growth performance of nursery pigs on commercial pig farms.

MATERIALS AND METHODS

Experimental animal feeding and sampling

The management and design of the experiment followed the animal care rules approved by the Animal Ethics Committee of Kasetsart University (Thailand). The Ethics Committee agreement number is U1-03970-2559. The study was carried out in a commercial pig farm, the study included eight-hundred weaned (6 weeks of age) and even-weight (Large White x Landrace x Duroc) piglets from a commercial pig farm in Saraburi Province, Thailand.

The pigs were assigned into two treatments with 8 replicates per treatment (50 pigs/pen). With an initial body weight of 8.54–8.58 kg, one group was routinely raised (Amox-Co) and the other group was raised without colistin (Amox-Phage). In the Amox-Co group, 300 ppm amoxycillin and 160 ppm colistin were added to the standard diet. This combination of antibiotics is commonly used in Thailand in feed for fattening pigs. In the Amox-Phage group (300 ppm amoxycillin and 1,000 mg bacteriophage cocktail/kg feed), the product contained a cocktail of bacteriophages of *Salmonella* (*S.* Typhimurium, *S.* Enteritidis, and *S.* Cholerasuis), *E. coli* (k88, F18, and Stx2e [ETEC] and EPEC, enteropathogenic *E. coli*), and *C. perfringens* types A and C. The titre of each bacteriophage in the bacteriophage cocktail was 10^9 pfu/g bacteriophage cocktail. Piglets were fed the same commercial feed and drank water ad libitum for a period of 6 weeks. Three piglets per pen (n = 12/treatment) were slaughtered, and the gastrointestinal tract was immediately removed. The duodenum, jejunum, ileum, and cecal contents were collected into 5 mL tube and immediately stored at -80 C until further microbial analysis. In these pigs, small sections were taken from the duodenum, jejunum, and ileum for histological examination to examined and analysed villus height and crypt depth.

Growth performance measurement and diarrhea incidence

The pigs were weighed individually at the start and at the end of experiment, recording the average daily feed intake (ADFI) and the ADG. The feed conversion ratio (FCR) was also determined. All piglets were checked for the consistency of fecal samples as an indicator of their health status. Fecal scores were based on diarrhea incidence (Perez et al., 2019), determined by using a consistency score system with three categories.

Table 1: DNA primer sequences for analyzed bacterial populations.

Bacteria		DNA sequence	Annealing temperature (C)	Source
Escherichia coli	F	5'-GACCTCGGTTTAGTTCACAGA-3'	60	Rekha et al., 2006
	R	5'-CACACGCTGACGCTGACCA-3'		
ETEC (F18)*	F	*5'*-GGTTTGACCACCTTTCAGTTGAGCAG-*3'*	63	Seo et al., 2018
	R	5'-TGGCACTGTAGGAGATACCATTCAGC-3'		
EHEC (hlyA) **	F	5'-ACGATGTGGTTTATTCTGGA-3'	50	(Kargar and Homayoon, 2015)
	R	5'-CTTCACGTCACCATACATAT-3'		

* Enterotoxigenic *E.coli*, ETEC
**Enterohemorrhagic *E.coli*, EHEC

Table 2: The Effect of Bacteriophage supplementation on the growth performance of nursery pigs.

Items	Amox-Co	Amox-Phage	*P*-value
Overall, 42 days			
Initial body weight, kg	8.58 ± 0.47	8.54 ± 0.48	0.874
Final body weight, kg	24.82 ± 1.17	25.25 ± 1.36	0.510
ADG (g)	396 ± 28	407 ± 28	0.454
ADFI (g)	587 ± 36	606 ± 38	0.326
FCR	1.48 ± 0.037	1.48 ± 0.040	0.742
BWG (kg)	16.25 ± 1.28	16.75 ± 1.38	0.466
Mortality+ Lost (%)	9.71 ± 2.29	7.46 ± 2.60	0.0875

Amox-Co: Amoxicillin + Colistin,
Amox-Phage: Amoxicillin + Bacteriophage.

DNA Isolation and 16S rRNA Gene Sequencing and Data Analysis

Total DNA was extracted from 0.25 gram of cecal content with a commercial extraction kit (QIAamp PowerFecal Pro DNA Kit, Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. DNA extracts were subjected to amplicon sequencing using Illumina. The sequencing library was constructed using a MetaVX Library Preparation Kit (GENEWIZ Inc., South Plainfield, NJ). The forward primers 5'-CCTACGGRRBGCASCAGKVRVGAAT-3' and the reverse primers 5'-GGACTACNVGGGTWTCTAATCC-3' amplicons that cover V3 and V4 regions of the 16s rRNA gene of bacteria were used in the PCR reaction. Next generation sequencing was conducted on the Illumina Miseq/Novaseq Platform (Illumina, San Diego, USA). The resulting 16S-rRNA sequences were imported and analysed by QIIME2 version 2022.11. The q2-diversity plugin was used to perform diversity analyses including alpha- and beta-diversity analyses. In addition, Bray Curtis and unweighted Unifrac metrics were used to construct distance matrices for beta-diversity. Linear discriminant analysis (LDA) effect size (LEfSe) was used to determine significant differences in abundance among different sample groups. LEfSe analysis was conducted using an LDA score of 2.

Real-Time PCR

PCR-based detection was used to confirm the presence/absence of *E. coli*, ETEC, and EHEC (enterohemorrhagic *E. coli*). Total DNA was extracted from 0.25 g of the intestinal contents with a commercial extraction kit (QIAamp PowerFecal Pro DNA Kit, Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. The DNA was used as a template for real-time PCR with specific primers (Table 1). Amplification was conducted using the HOT FIREPol® EvaGreen® qPCR Mix Plus (Solid Biodye, Inc.) at the following conditions: initially 15 min at 95 C, 30 cycles with 15 s at 95 C, and 60 s at 60 C for product amplification. The number of bacteria were reported as log cfu/g of digesta.

Statistical analysis

Data were subjected to statistical analysis with Student t-test using the SAS software (version 9.4; SAS Institute Inc., Cary, NC) with pigs being the experimental units. P-value ≤0.05 is considered statistically significant.

RESULTS

Growth performance and piglet diarrhea

Table 2 showed the effect of bacteriophages used to replace

Table 3: The Effect of Bacteriophage supplementation on fecal score of nursery pigs.

Fecal score	Amox-Co	Amox-Phage	*P*-value
Week 1	1.38 ± 0.24	1.28 ± 0.14	0.325
Week 2	1.40 ± 0.21	1.38 ± 0.13	0.856
Week 3	1.46 ± 0.14	1.45 ± 0.18	0.842
Week 4	1.57 ± 0.22	1.61 ± 0.24	0.708
Week 5	1.56 ± 0.11	1.42 ± 0.22	0.121
Week 6	1.39 ± 0.05[a]	1.24 ± 0.13[b]	0.015

Amox-Co: Amoxicillin + Colistin, Amox-Phage: Amoxicillin + Bacteriophage.
Fecal score: 0= normal faeces, 1= soft faeces, 2 = mild diarrhoea, and 3 = severe diarrhoea [a,b] Means in a row with no common superscripts differ (p≤0.05)

Table 4: The Effect of Bacteriophage supplementation on small intestinal morphology

Items	Amox-Co	Amox-Phage	P value
Duodenum			
Villus height (µm)	503.1 ± 105.9	500.3 ± 115.5	0.9127
Crypt depth (µm)	310.9 ± 71.3	327.6 ± 83.3	0.3637
VH:CD ratio	1.63 ± 0.087[a]	1.55 ± 0.082[b]	<0.0001
Jejunum			
Villus height (µm)	535.7 ± 75.58[a]	410 ± 75.37[b]	<0.0001
Crypt depth (µm)	243.6 ± 43.53[a]	212.4 ± 25.63[b]	0.0088
VH:CD ratio	2.218 ± 0.14	1.9234 ± 0.15	<0.0001
Ileum			
Villus height (µm)	449.4 ± 106.7	417.3 ± 54.23	0.2383
Crypt depth (µm)	226.9 ± 56.22	215.9 ± 43.33	0.4906
VH:CD ratio	1.98 ± 0.16	1.96 ± 0.16	0.6433

Amox-Co: Amoxicillin + Colistin, Amox-Phage: Amoxicillin + Bacteriophage. VH:CD: Villus height (µm)/ Crypt depth (µm)
[a,b] Means in a row with no common superscripts differ (p≤0.05)

Table 5: The Effect of Bacteriophage supplementation on *E. coli*, ETEC (F18) and EHEC population (log CFU/g) in nursery pigs.

E. coli population	Amox-Co	Amox-Phage	*P*-value
Total *E. coli*			
Duodenum	10.36 ± 1.08	10.51 ± 0.67	0.635
Jejunum	9.74 ± 0.70	9.80 ± 0.74	0.543
Ileum	10.55 ± 1.12	11.14 ± 1.56	0.079
Cecum	9.83 ± 0.20	9.91 ± 0.44	0.190
ETEC (F18)			
Duodenum	ND	ND	
Jejunum	ND	ND	
Ileum	ND	ND	
Cecum	ND	ND	
EHEC			
Duodenum	ND	ND	
Jejunum	ND	ND	
Ileum	ND	ND	
Cecum	ND	ND	

Amox-Co: Amoxicillin + Colistin, Amox-Phage: Amoxicillin + Bacteriophage.
ND = Non-detect

antibiotics (colistin) (Amox-Phage group) in nursery pigs for 6 weeks. The ADG, ADFI, and FCR did not differ compared with the Amox-Co group. Furthermore, the mortality of piglets in the bacteriophage-supplemented group was 7.46%, while the mortality in the Amox-Co group was 9.71%. The effect of bacteriophage supplementation on the fecal score of nursery pigs is shown in Table 3, represented by the diarrhea incidence. The fecal scores were not significantly different between the groups during weeks 1–5. At the end of the experiment (week 6), there were lower fecal scores in the Amox-Phage group ($p < 0.05$).

Morphology of the small intestine

The effect of bacteriophage supplementation on small intestinal morphology is shown in Table 4. The villus, jejunum, and ileum of the Amox-Co group were significantly higher than the Amox-Phage group ($p < 0.05$); however, the height of the duodenum was not different between the groups. The crypt depth was significantly higher in the Amox-Co group than in the Amox-Phage group ($p < 0.05$). Representative images of hematoxylin-eosin-stained small intestine are shown in Figure 1. The Amox-Phage group had a normal appearance with regular intestinal villus structure when compared with the Amox-Co group; there was no obvious damage. In the duodenum, the Amox-Co group had epithelial shedding. In addition, the duodenum of the Amox-Phage group contained more goblet cells (black arrow) than the Amox-Co group.

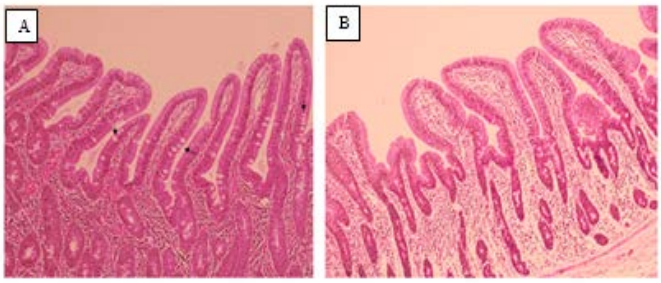

Figure 1: Micrograph (100X) intestinal morphology of nursery pigs. A = This micrograph was presented higher numbers of Goblet cells in duodenum of bacteriophage supplementation (Amox-Phage) compared to Amox-Co group (B). Goblet cell (colorless and round-cup shaped) were present in villi of the small intestine (black arrow).

Gut microbiota richness and diversity

Overall, 2,171,459 quality-filtered sequences from the cecum of nursery pigs showed similarity to sequences in the SILVA database. There was no significant difference ($p > 0.05$) in species richness, as represented by the Observed_species values in the two treatments (Fig. 2A). There was a high Shannon diversity index value in the Amox-Phage group; however, there was no significant difference ($p > 0.05$) in species diversity compared with the Amox-Co group. The evenness of the cecal microbiota, as evaluated by Pielou's evenness index, changed but not significantly ($p > 0.05$) throughout the experiment.

Cecal microbiota composition and Real-Time PCR detection

We observed the effects of dietary bacteriophage supplementation on the cecal microbial composition of piglets at different taxonomic levels. The overall bacterial compositions of the cecal samples at the phylum level are shown in Figure 2B. Firmicutes was predominant in all groups, with a relative abundance of 92.89% and 95.74% in the Amox-Phage and Amox-Co groups, respectively. The relative abundance of Bacteroidetes increased slightly upon the addition of bacteriophages (1.41% in the Amox-Phage group and 0.28% in the Amox-Co group). The Proteobacteria phylum was predominant ($p < 0.05$) in the Amox-Phage group (0.84%) compared with the Amox-Co group (0.06%). At the family level, LEfSe analysis showed a remarkable difference between the groups in terms of the relative abundances of cecal microbiota (Fig. 2C). The relative abundances of the Lactobacillaceae, Pseudomonadaceae, and Enterobacteriaceae families were significantly higher ($p < 0.05$) in the Amox-Phage group, while the relative abundances of the Leuconostocaceae, Ruminococcaceae, and Anaerovoracaceae families were significantly higher in the Amox-Co group. There were eight genera with significantly dominant bacterial populations in the Amox-Phage group: *Pediococcus*, *Vagococcus*, *Enterococcus*, *Escherichia*, *Shigella*, *Pseudomonas*, *Aerococcus*, and *Clostridium*_sensu_stricto_13.

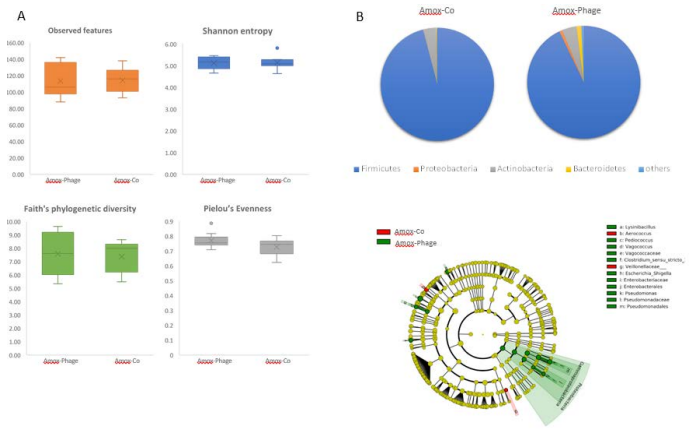

Figure 2: (A) Diversity parameters. Parameters including index of Observed features, Shannon entropy, Faith's phylogenetic diversity and Pielou's Evenness. (B) Relative abundant phyla. (C) The histogram of the linear discriminant analysis (LDA) score illustrating the differential abundant. Amox-Co: Amoxicillin + Colistin, Amox-Phage: Amoxicillin + Bacteriophage.

Table 5 showed the quantification/ detection of *E. coli*, ETEC, and EHEC. The *E. coli* population in the duode-

num, jejunum, ileum, and cecum did not differ between groups ($p > 0.05$), and ETEC (F18) and EHEC were not detected.

DISCUSSION

In pig production, stressful changes in weaned pigs leads to immune system dysfunctions and decreased piglet health and growth performance (Campbell et al., 2013). The findings of this study are consistent with previous reports (Yan et al., 2012; Oh et al., 2022). Supplementing bacteriophages in the diet of nursery pigs to replace colistin did not affect the ADFI, the ADG, or the FCR. These results are different from those of Kim et al. (2017), who stated that the ADG or the G:F ratio were improved after bacteriophage supplementation. The current study revealed that bacteriophage supplementation did not affect the growth performance of pigs, even when they lacked the effects of colistin. Moreover, pigs fed bacteriophages had a significantly decreased diarrhea incidence compared with the antibiotic group at the late period of the experiment. A fecal score greater than 3 is defined as a clinical sign of diarrhea (Luise et al., 2019; Perez et al., 2019). Kim et al. (2017) reported a reduced fecal diarrhea score in weaned pigs fed bacteriophages; the bacteriophage cocktail resulted in a greater fecal score compared with the control.

We did not infect pigs with ETEC in this study because the experiment was performed on a commercial farm. *E. coli*, ETEC, and EHEC were quantified in the digesta by using real-time PCR. The ETEC and EHEC were not detected in the control or Amo-Phage groups. Because *E. coli* occurs as part of the normal intestinal bacterial flora and has hundreds of serotypes, we also measured total *E. coli* (based on 16S rRNA); the *E. coli* counts did not differ between the groups. This finding suggests that pigs treated with bacteriophages show similar total *E. coli* counts in the digestive tract compared with those fed colistin (Yan et al., 2012; Kim et al., 2014).

The high-throughput 16S rRNA sequencing was used to evaluate the abundance and diversity of the cecal microbial community in pigs. The diversity of the Amox-Phage group was higher than that of the Amox-Co group ($p > 0.05$). These findings indicate that the use of bacteriophages to replace colistin can enhance microbial diversity (Abeles et al., 2016; Correa-Fiz et al., 2019). Overall, long-term bacteriophage supplementation (more than 6 weeks) can improve gut microbial diversity and increase species richness and OTUs. In the cecal gut microbiota of piglets, Firmicutes (92.89%) was the dominant bacterial phylum; however, Proteobacteria, which contains several genera with known animal pathogens—*Escherichia*, *Shigella*, *Salmonella*, and *Yersinia*—was not be reduced in the Amox-Phage group (colistin replacement). The bacteriophages against *Shigella* and *Yersinia* as well as other *E. coli* and *Salmonella* serotypes were not included in the bacteriophage cocktail used in this study, and therefore, Proteobacteria was the dominant phylum. Song et al. (2019) stated that a characteristic feature of bacteriophages is their ability to recognize proteins present on pathogenic strains; hence, they can only kill the pathogen that they can recognize. In a recent metagenomic analysis of the fecal microbiota, the genera *Prevotella*, *Sutterella*, and *Campylobacter*, and the family Fusobacteriaceae dominated in diarrheic piglets (Yang et al., 2017); we did not observe these changes in the Amox-Phage group. A few members of Bacteroidetes showed decreased relative abundances in the cecum of the Amox-Co group (control); these bacteria provide protection against pathogens and supply nutrients to the microbiota (Zafar and Saier, 2019).

The Amox-Phage group displayed much higher relative abundances of the families Lactobacillaceae and Enterobacteriaceae. This explains the significant increase in the abundances of the genera *Pediococcus* and *Enterococcus* in this group. Lactobacillaceae is considered one of the most promising replacements and, therefore, represents a safe alternative to substitute antibiotics in pigs (Dowarah et al., 2017). According to recent studies, the gut microbiota of healthy piglets has a higher abundance of Lactobacillaceae, the members of which regulate maturation of the innate immune system after weaning (Dou et al., 2017). In addition, Enterobacteriaceae (including the genus *Enterococcus*) includes commensal gut bacteria isolated for food, feed, and probiotic use (Hanchi et al., 2018). The genus *Pediococcus*, which increased significantly in the Amox-Phage group, could modulate microbial communities related to the intestinal health of piglets (Wang et al., 2019). In their recent review, Lekang et al. (2022) discussed how amoxicillin disturbs the overall bacterial diversity and richness. This phenomenon may clarify how the bacteriophage cocktail altered the gut microbiota.

The changes in small intestinal morphology are indications of gut health and digestive efficiency of pigs. In the present study, enterocyte proliferation was not accelerated by bacteriophage supplementation. Researchers have observed that the intestinal morphology and proliferation of epithelial cells that increase the villus height are impaired by dominant pathogenic bacteria due to partial pathogen colonization and damage to villus cells (Mourao et al., 2005; Kitamoto et al., 2016). Shorter villi were observed than those found in the jejunum and ileum in the Amox-Phage group, probably because most phages are inhibited by intestinal glycan in the mucus layer (Green et al., 2021). Kim et al. (2017) reported that supplementation with bacteriophages and probiotics increased the villus height in

weaned pigs. These results, however, are somewhat different from the findings of our study, where the structural variables of the villus did not change in the Amox-Phage group. A normal intestinal villus structure and no obvious damage were observed in the Amox-Phage group. Moreover, the number of goblet cells increased in the duodenum because the abundance of *Lactobacillus* in the Amox-Phage group induced an intestinal mucosal immune response, and mucus is secreted by intestinal goblet cells (Tassell et al., 2011; Peng et al., 2021). The density of goblet cells we found in this study are similar to those observed by Jang et al. (2014). Therefore, bacteriophages and antibiotics improve different aspects of nursery pig performance, but supplementation with a bacteriophage cocktail and amoxicillin have a greater ability to reduce diarrhea than supplementation with antibiotics alone.

CONCLUSION

Dietary supplementation with amoxicillin and a bacteriophage cocktail controlled the *E. coli* population and altered the abundance of Lactobacillaceae in the cecum. This treatment may also help to improve intestinal morphology, but it does not improve the growth performance of nursery pigs.

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CONFLICT OF INTEREST

No conflict of interest.

NOVELTY STATEMENT

The main objective of this paper is to find out the influence of bacteriophages on the diarrhea score and *E. coli* intestinal colonization, cecal microbiota composition and growth performance, performance in commercial pigs is published for the first time.

AUTHORS CONTRIBUTION

KP - carried out the experiment, preparation of an article for publication. ST – performed the study of intestinal microbiota.

RC - designed the model, verified the analytical methods, helped supervise the project. BC - helped supervise the project. SK – Microbiota data analysis. LW- supervised the project, preparation of an article for publication.

REFERENCES

Abeles SR, Jones MB, Santiago-Rodriguez TM, Ly M, Klitgord N, Yooseph S, Nelson KE, Pride DT (2016). Microbial diversity in individuals and their household contacts following typical antibiotic courses. Microbiome.4:39. https://doi.org/10.1186/s40168-016-0187-9

Bogere P, Choi YJ, Heo J (2019). Probiotics as alternatives to antibiotics in treating post-weaning diarrhoea in pigs: review paper. S. Afr. J. Anim. Sci. 49:403-416. https://doi.org/10. 4314/sajas.v49i3.1

Bruun T, Sørensen G, Forshell LP, Jensen T, Nygård K, Kapperud G, Lindstedt BA, Berglund T, Wingstrand A, Petersen RF, Muller L, Kjelsø C, Ivarsson S, Hjertqvist M, Löfdahl S, Ethelberg S (2009) An outbreak of Salmonella Typhimurium infections in Denmark, Norway and Sweden, 2008. Euro Surveill. 14(10): 1– 6. https://doi.org/10.2807/ese.14.10.19147-en.

Campbell JM, Crenshaw JD, Polo J (2013). The biological stress of early weaned piglets. J. Anim. Sci. Biotechnol. 30;4(1):19. https://doi.org/10.1186/2049-1891-4-19

Correa-Fiz F, Gonçalves dos Santos JM, Illas F, Aragon V (2019). Antimicrobial removal on piglets promotes health and higher bacterial diversity in the nasal microbiota. Sci. Rep. 9(6545): 1-9. https://doi.org/10.1038/s41598-019-43022-y

Dou S, Gadonna-Widehem P, Rome V, Hamoudi D, Rhazi L, Lakhal L, Larcher T, Bahi-Jaber N, Pinon-Quintana A, Guyonvarch A, Huërou-Luron IL, Abdennebi-Najar L (2017). Characterisation of early-life fecal microbiota in susceptible and healthy pigs to post-weaning diarrhoea. PLoS. 10;12(1): e0169851. https://doi.org/10.1371/journal.pone.0169851

Dowarah R, Verma AK, Agarwal N, Patel BHM, Singh P (2017). Effect of swine based probiotic on performance, diarrhoea scores, intestinal microbiota and gut health of grower-finisher crossbred pigs. Livest. Sci. 195: 74-79. https://doi.org/10.1016/j.livsci.2016.11.006.

Fairbrother JM, Nadeau E, Gyles CL (2005). *Escherichia coli* in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. Anim. Health Res. Rev. 6(1):17-39. https://doi.org/10.1079/ahr2005105

Ghaisas S, Maher J, Kanthasamy A (2016). Gut microbiome in health and disease: Linking the microbiome-gut-brain axis and environmental factors in the pathogenesis of systemic and neurodegenerative diseases. Pharmacol Ther. 158:52-62. https://doi.org/10.1016/j.pharmthera.2015.11.012

Green SI, Gu Liu C, Yu X, Gibson S, Salmen W, Rajan A, Carter HE, Clark JR, Song X, Ramig RF, Trautner BW, Kaplan HB, Maresso AW (2021). Targeting of mammalian glycans enhances phage predation in the gastrointestinal tract. mBio. 12(1). https://doi.org/10.1128/mBio.03474-20.

Hanchi H, Mottawea W, Sebei K, Hammami R (2018). The genus Enterococcus: between probiotic potential and safety concerns-an update. Front Microbiol. 3(9):1791. https://doi.org/10.3389/fmicb.2018.01791.

Heo JM, Opapeju FO, Pluske JR, Kim JC, Hampson DJ, Nyachoti CM (2013). Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhoea without using in-feed antimicrobial compounds. J. Anim. Physiol. Anim. Nutr. 97:207-237. https://doi.org/10.1111/j.1439-0396.2012. 01284.x

Hermann-Bank ML, Skovgaard K, Stockmarr A, Strube ML, Larsen N, Kongsted H, Ingerslev HC, Mølbak L, Boye M (2015). Characterization of the bacterial gut microbiota of piglets suffering from new neonatal porcine diarrhoea. BMC Vet Res. 11(139): 1– 19. https://doi.org/10.1186/s12917-015-0419-4

Jang MH, Kweon MN, Iwatani K, Yamamoto M, Terahara K, Sasakawa C, Suzuki T, Nochi T, Yokota Y, Rennert PD, Hiroi T, Tamagawa H, Iijima H, Kunisawa J, Yuki Y, Kiyono H (2004). Intestinal villous M cells: an antigen entry site in the mucosal epithelium. Proc. Natl. Acad. Sci .U S A. 101(16): 6110-5. https://doi.org/doi: 10.1073/pnas.0400969101.

Kargar M, Homayoon M (2015). Prevalence of shiga toxins (stx1, stx2), eaeA and hly genes of Escherichia coli O157:H7 strains among children with acute gastroenteritis in southern of Iran. Asian Pac J Trop Med. (1):24-8. https://doi.org/10.1016/S1995-7645(14)60182-6

Kim KH, Ingale SL, Kim JS, Lee SH, Lee JH, Kwon IK, Chae BJ (2014). Bacteriophage and probiotics both enhance the performance of growing pigs but bacteriophage are more effective. Anim. Feed Sci. Technol. 196: 88–95. https://doi.org/10.1089/fpd.2013.1600

Kim JS, Hosseindoust A, Lee SH, Choi YH, Kim MJ, Lee JH, Kwon IK, Chae BJ (2017). Bacteriophage cocktail and multi-strain probiotics in the feed for weanling pigs: effects on intestine morphology and targeted intestinal coliforms and Clostridium. Animal. 11(1):45-53. https://doi.org/10.1017/S1751731116001166

Lee CY, Kim SJ, Park BC, Han JH (2016). Effects of dietary supplementation of bacteriophages against enterotoxigenic *Escherichia coli* (ETEC) K88 on clinical symptoms of post-weaning pigs challenged with the ETEC pathogen. J. Anim. Physiol. Anim. Nutr. (Berl) 101(1):88-95. https://doi.org/10.1111/jpn.1251

Lekagul, A, Tangcharoensathien V, Liverani M (2021). Understanding antibiotic use for pig farming in Thailand: a qualitative study. Antimicrob. Resist. Infect. Control. 10(3). https://doi.org/10.1186/s13756-020-00865-9

Lekang K, Shekhar S, Berild D, Petersen FC, Winther-Larsen HC (2022). Effects of different amoxicillin treatment durations on microbiome diversity and composition in the gut. PLoS One. 2022 Oct 27;17(10): e0275737. https://doi.org/ 10.1371/journal.pone.0275737

Lo BC, Chen GY, Núñez G, Caruso R (2021). Gut microbiota and systemic immunity in health and disease. Int. Immunol. 33(4):197-209. https://doi.org/10.2807/0.1093.

Luise D, Lauridsen C, Bosi P, Trevisi P. (2019). Methodology and application of Escherichia coli F4 and F18 encoding infection models in post-weaning pigs. J. Animal. Sci. Biotechnol. 10(53): 1-20. https://doi.org/10.1186/s40104-019-0352-7

Mourão JL, Pinheiro V, Alves A, Guedes CM, Pinto L, Saavedra MJ, Spring P, Kocher A (2006) Effect of mannan oligosaccharides on the performance, intestinal morphology and cecal fermentation of fattening rabbits. Anim. Feed Sci. Technol. 126: 107-120. https://doi.org/10.1016/j.anifeedsci.2005.06.009.

Nagao-Kitamoto H, Kitamoto S, Kuffa P, Kamada N (2016) Pathogenic role of the gut microbiota in gastrointestinal diseases. Intest. Res. 14(2):127-38. https://doi.org/10.5217/ir.2016.14.2.127.

Nagy B, Fekete PZ (2005). Enterotoxigenic *Escherichia coli* in veterinary medicine. Int J Med Microbiol. 295(6-7):443-454. https://doi.org/10.1016/j.ijmm.2005.07.003

National Research Council (NRC) (2012) Nutrient Requirements of Swine. 11th Edition, National Academies Press, Washington DC. 420 pp.

Oh SH, Jang JC, Lee CY, Han JH, Park BC (2022). Direct-fed Enterococcus faecium plus bacteriophages as substitutes for pharmacological zinc oxide in weanling pigs: effects on diarrheal score and growth. Anim. Biosci. 35(11):1752-1759. https://doi.org/10.5713/ab.22.0262

Peng Y, Wang ZN, Xu AR, Fang ZF, Chen SY, Hou XT, Zhou ZQ, Lin HM, Xie JX, Tang XX, Wang DY, Zhong NS (2022). Mucus hypersecretion and ciliary impairment in conducting airway contribute to alveolar mucus plugging in idiopathic pulmonary fibrosis. Front Cell Dev. Biol. 9:810842. https://doi.org/10.3389/fcell.2021.810842.

Perez-Calvo E, Wicaksono AN, Canet E, Daulton E, Waldemar E, Ulrich H, Viviane V, Pietro C, Covington JA (2019). The measurement of volatile organic compounds in faeces of piglets as a tool to assess gastrointestinal functionality. Biosystemseng. 184: 122-129. https://doi.org/10.1016/j.biosystemseng.2019.06.005

Seo BJ, Song ET, Lee K, Kim JW, Jeong CG, Moon SH, Son JS, Kang SH, Cho HS, Jung BY, Kim WI (2018). Evaluation of the broad-spectrum lytic capability of bacteriophage cocktails against various Salmonella serovars and their effects on weaned pigs infected with Salmonella *Typhimurium*. J. Vet. Med. Sci. 6;80(6):851-860. https://doi.org/ 10.1292/jvms.17-0501

Song S, Guo Y, Kim JS, Wang X, Wood TK (2019). Phages mediate bacterial self-recognition. Cell Rep. 16;27(3):737-749.e4. https://doi.org/10.1016/j.celrep.2019.03.070. PMID: 30995473

Van Tassell ML, Miller MJ (2011). Lactobacillus adhesion to mucus. Nutrients. 3(5):613-36. https://doi.org/10.3390/nu3050613.

Wang S, Yao B, Gao H, Zang J, Tao S, Zhang S, Huang S, He B, Wang J (2019). Combined supplementation of Lactobacillus fermentum and *Pediococcus acidilactici* promoted growth performance, alleviated inflammation, and modulated intestinal microbiota in weaned pigs. BMC Vet. Res. 15, 239. https://doi.org/10.1186/s12917-019-1991-9

Yan L, Hong SM, Kim IH (2012). Effect of bacteriophage supplementation on the growth performance nutrient digestibility blood characteristics and fecal microbial shedding in growing pigs. Asian-Aust. J. Anim. Sci 25(10): 1451–1456. https://doi.org/10.5713/ajas.2012.12253

Yang Q, Huang X, Zhao S, Sun W, Yan Z, Wang P, Li S, Huang W, Zhang S, Liu L, Gun S (2017). Structure and function of the fecal microbiota in diarrheic neonatal piglets. Front Microbiol. 8(502): 1-13. https://doi.org/10.3389/fmicb.2017.00502

Zafar H, Saier MH Jr (2019). Gut bacteroides species in health and disease. Gut Microbes. 13(1):1-20. https://doi.org/10.1080/19490976.2020.1848158