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Effect of Bacteriophage Supplementation on the Growth Performance, Nutrient Digestibility, Blood Characteristics, and Fecal Microbial Shedding in Growing Pigs

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BSTRACT: A total of 144 ((Duroc×Yorkshire)×Landrace)) pigs with an average initial BW of 28.85±0.63 kg were used in this 6-wk growth trial. Pigs were randomly allotted to 1 of 4 treatments in a completely random block design. Each dietary treatment consisted of 9 replicate pens, with 4 pigs per replicate. Dietary treatments included: i) NC (basal diet), ii) PC (NC+apramycin 0.5 g/kg), iii) BPT1 (NC+bacteriophage 0.25 g/kg) and iv) BPT2 (NC+bacteriophage 0.5 g/kg). The inclusion of antibiotics and bacteriophages did not affect the ($p>0.05$) ADG, ADFI and G:F compared with the basal diet. Dietary antibiotics and bacteriophages supplementation led to a higher ($p<0.05$) DM digestibility than the NC treatment. Pigs fed the bacteriophage supplemented diet increased ($p<0.05$) the N digestibility compared with those fed NC treatment. Supplementation of antibiotics led to a higher ($p<0.05$) energy digestibility than the NC treatment. No difference ($p>0.05$) was observed in the RBC, WBC, lymphocyte concentration and fecal moisture among treatments. Pigs fed PC and BPT2 treatments reduced ($p<0.05$) the *E. coli* concentration compared with those fed NC treatment. The inclusion of BPT2 treatment led to a higher ($p<0.05$) lactobacillus concentration compared with NC and PC treatment. Dietary antibiotic and bacteriophage supplementation reduced ($p<0.05$) the *Salmonella* concentration compared with NC treatment. In conclusion, our study suggested that bacteriophage at the level of 0.5 g/kg could be used as an antibiotics alternative for growing pigs. (**Key Words:** Bacteriophages, Growth, Digestibility, Fecal Microbes, Growing Pigs)

INTRODUCTION

With the ban of antibiotic utilization in livestock for growth promotion, bacterial infection of the animal became emerging problem in pig industry. Thus, there was increased interest of finding better antibiotic alternatives against bacterial infection and subsequently promoting the growth performance in pigs (Yan et al., 2010; Yan et al., 2011a;b; Yan et al., 2012). Among these alternative, bacteriophages had received great attention in the discussion about developing suitable alternative for antibiotics growth promoter in livestock industry (Jamalludeen et al., 2009; Lee and Harris, 2001)

As demonstrated elsewhere, bacteriophages are non-harzardous self-replicating agent that can infect and multiply in bacteria to prevent bacterial diseases. Smith and Huggins (1982) have previously suggested that the treatment of *E. coli* infected mice with phages was more effective than treatment with antibiotics. Lee and Harris (2001) demonstrated that administration of a single broad-spectrum anti-Salmonella phage to *Salmonella* challenged young pigs could reduce *Salmonella* concentration in several tissues. Jamalludeen et al. (2009) also suggested that the inclusion of phages could prevent the *E. coli* induced diarrhea in weaned pigs. However, most of those previous studies were conducted under bacterial challenge condition, only a limited research had been conducted under normal physiological state. Recently, our previous study reported that the inclusion of bacteriophgaes could also benefit the laying hens in egg production and egg quality under normal physiological state (Zhao et al., 2012).

Thus, the aim of the present study was to evaluate the effects of bacteriophages on growth performance, nutrient digestibility, blood characteristics, fecal microbial shedding and fecal moisture in growing pigs, we hypothesized the inclusion of bacteriophages could also benefit the animal performance under normal state.

MATERIALS AND METHODS

The experimental protocols were approved by the

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Animal Care and Use Committee of Dankook University.

Experimental design, animals, and housing

Bacteriophages used in our study contain Salmonella gallinarum, Salmonella typhimurium, and S. Enteritidis. The concentration of bacteriophages is 10^8 plaque forming unit per gram. A total of 144 ((Duroc×Yorkshire)× Landrace)) pigs with an average initial BW of 28.85±0.63 kg were used in this 6-wk growth trial. Pigs were randomly allotted to 1 of 4 treatments in a completely random block design. The bacteriophage was administrated by replacing the same amount of corn. Each dietary treatment consisted of 9 replicate cages, with 4 pigs per replicate (two barrows and two gilts). Dietary treatments included: i) NC (basal diet), ii) PC (NC+apramycin 0.5 g/kg), iii) BPT1 (NC+ bacteriophage 0.25 g/kg) and iv) BPT2 (NC+bacteriophage 0.5 g/kg). Diets (Table 1) were formulated to meet or exceed the nutrient requirements recommended by NRC (1998). Pigs were housed in an environmentally controlled, slatted-floor facility in 36 adjacent pens (1.8×1.8 m) at the pig farm of Dankook University. All pigs were provided with *ad libitum* access to feed and water through a self-feeder and nipple drinker, respectively, throughout the experiment. The target room temperature and humidity were 25°C and 60%, respectively.

Table 1. Feed composition of control diet (as-fed basis)

Item	Diet
Ingredient (%)	
Corn	49.30
wheat	10.00
Rice bran	1.42
Wheat bran	2.00
Soybean meal	23.72
DDGS (dried distiller's grain with solubles)	5.00
Limestone	0.22
Animal fat	2.50
Molasses	3.00
Salt	0.30
Choline (50%)	0.07
Methionine (99%)	0.04
L-lysine	0.26
DCP (Dicalcium phosphate)	1.46
Threonine (100%)	0.02
Mineral preminx[1]	0.55
Vitamin premix[2]	0.14
Total	100.00
Calculated composition (%)	
Crude protein	18.0
Crude fat	5.39
Crude ash	5.26
L-lysine	0.97
Calcium	0.70
Phosphorus	0.65

[1] Provided per kg of complete diet: vitamin A, 4,000 IU; vitamin D_3, 800 IU; vitamin E, 171 IU; vitamin K, 2 mg; riboflavin, 4 mg; niacin, 20 mg; thiamine, 4 mg; d-pantothenic, 11 mg; choline, 166 mg; biotin, 0.08 mg; and vitamin B_{12}, 16 μg.

[2] Provided per kg of complete diet: Cu (as $CuSO_4 \cdot 5H_2O$), 15 mg; Fe (as $FeSO4 \cdot 7H_2O$), 80 mg; Zn (as $ZnSO_4$), 56 mg; Mn (MnO_2), 74 mg; I (as KI), 0.3 mg; Co (as $CoSO_4 \cdot 5H_2O$), 0.5 mg; and Se (as $Na_2SeO_3 \cdot 5H_2O$), 0.4 mg.

Sampling and measurements

Pig weights were measured at the beginning and the end of the experiment period, feed consumption were recorded on a pen basis during the experiment to calculate ADG, ADFI, and G:F. Chromium oxide was added to the diet as an indigestible marker at 0.20% of the diet for 7 d prior to fecal collection at the 6th wk for calculation of DM, N, and energy digestibility. Fecal grab samples were collected at random from at least 2 pigs in each pen (1 gilt and 1 barrow) at the end of the study. All feed and feces samples were stored immediately at -20°C until analysis. All the feed and fecal samples were freeze-dried and finely ground to be able to pass through a 1-mm screen. The determination of DM, N, and energy digestibility were conducted in accordance with the methods established by the AOAC (2000). Chromium levels were determined via UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan). The gross energy was determined by measuring the heat of combustion in the samples using a Parr 6100 oxygen bomb calorimeter (Parr instrument Co., Moline, IL, USA). The CATTD of DM, N, and energy were calculated using indirect methods described by Williams et al. (1962).

Two pigs were randomly selected from each pen (1 gilt and 1 barrow) and bled via jugular venipuncture at the beginning of the experiment (0 d). The same pigs were bled at the end (35 d) of the experiment. Blood samples were collected into vacuum tubes containing K_3EDTA (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) to obtain whole blood. The red blood cells (RBC), white blood cells (WBC) and lymphocyte counts of whole blood samples were determined using an automatic blood analyzer (ADVIA 120, Bayer, Tarrytown, NY, USA).

At the end of experiment, fecal samples were collected via massaging the rectum from 2 pigs randomly selected from each pen (1 gilt and 1 barrow) and pooled and placed on ice for transportation to the laboratory, where analysis was immediately carried out. The composite fecal sample (1 g) from each pen was diluted with 9 ml of 10 g/L peptone broth (Becton, Dickinson and Co., Rutherford, NJ, USA) and homogenized. Viable counts of bacteria in the fecal samples were then conducted by plating serial 10-fold

Table 2. Effects of bacterphage on growth performance in growing pigs[1]

Items	NC	PC	BPT1	BPT2	SE[2]
ADG (g)	459	464	455	472	20
ADFI (g)	1,284	1,231	1,294	1,272	46
G/F	0.357	0.377	0.352	0.371	0.009

[1] NC = Basal diet (negative control); PC = Basal diet+22 ppm tylosin (positive control); BPT1 = NC+0.025% bacterphage; BPT2 = NC+0.05% bacterphage.
[2] Standard error.

Table 3. The effects of bacterphage on nutrient digestibility in growing pigs[1]

Items (%)	NC	PC	BPT1	BPT2	SE[2]
Dry matter	77.40^{b}	80.08^{a}	79.34^{a}	79.57^{a}	0.56
Nitrogen	77.00^{b}	78.43^{ab}	80.13^{a}	79.22^{a}	0.59
Energy	76.63^{b}	79.22^{a}	77.79^{ab}	78.46^{ab}	0.67

[1] NC = Basal diet (negative control); PC = Basal diet+22 ppm tylosin (positive control); BPT1 = NC+0.025% bacterphage; BPT2 = NC+0.05% bacterphage.
[2] Standard error.
[a,b] Means in the same row with different superscripts differ ($p<0.05$).

dilutions (in 10 g/L peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI, USA) and lactobacilli medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate the *E. coli* and *Lactobacillus*, respectively. The lactobacilli medium III agar plates were then incubated for 48 h at 39°C under anaerobic conditions. The MacConkey agar plates were incubated for 24 h at 37°C. The *E. coli* and *Lactobacillus* colonies were counted immediately after removal from the incubator. For *Salmonella*, the serially diluted peptone broth tubes were incubated overnight at 37°C, after which 1 ml was transferred to 9 ml of tetratinate broth (Neogen Corporation, Lansing, MI, USA) and then incubated for 48 h at 42°C. From these tubes, 1 ml was used to inoculate 9 ml of Rappaport Vassiliadis broth (Neogen Corporation) and incubated for 48 h at 42°C. The Rappaport was used to inoculate XLT4 plates for *Salmonella* isolation, and the *Salmonella* was then identified using LIS and TSI agar tubes (Difco Laboratories). Moreover, pigs excreta samples were collected and placed in aluminum foil cups. The aluminum foil cups were weighed and placed in a drying oven at 100°C for 24 h and then reweighed to calculate moisture loss. The incidence of diarrhea in piglets was observed and recorded 3 times (8:00, 14:00 and 20:00) per day during the study.

To assess the severity of diarrhea, fresh faeces from all pigs were scored during d 5 to 10 (most serious period) by determining the moisture content according to the method of Hart and Dobb (1988). Briefly, scores were 0, normal, firm faeces; 1, possible slight diarrhea; 2, definitely unformed, moderately fluid faeces; or 3, very watery and frothy diarrhea. The occurrence of diarrhea was defined as maintaining faecal scores of 2 and 3 for 2 consecutive days.

Statistical analyses

Data were analyzed by ANOVA using the General Linear Models (GLM) procedure of SAS (SAS Institute, 1996), with the pen being defined as the experimental unit. Differences among treatments were separated by Duncan's multiple range test. The results were expressed as the least squares means and SE. Probability values less than 0.05 were considered significant.

RESUTLS

Growth performance

The inclusion of antibiotics and bacteriophages did not affect the ($p>0.05$) ADG, ADFI and G:F ratio compared with the basal diet (Table 2).

Nutrient digestibility

Dietary antibiotics and bacteriophages supplementation led to a higher ($p<0.05$) DM digestibility than the NC treatment (Table 3). Pig fed the bacteriophages supplemented diet increased ($p<0.05$) the N digestibility compared with those fed NC treatment. Supplementation of antibiotics led to a higher ($p<0.05$) energy digestibility than the NC treatment. No difference ($p>0.05$) was observed among antibiotic treatment and bacteriophages supplemental diet.

Blood characteristics

No difference ($p>0.05$) was observed on the RBC, WBC, and lymphocyte concentration among treatments (Table 4).

Fecal microbial shedding and fecal moisture

Pig fed PC and BPT2 treatment reduced ($p<0.05$) the *E. coli* concentration compared with those fed NC treatment (Table 5). The inclusion of BPT2 treatment led to a higher ($p<0.05$) lactobacillus concentration compared with NC and PC treatment. Dietary antibiotic and bacteriophages supplementation significantly reduced ($p<0.05$) the salmonella concentration compared with NC treatment. No difference ($p>0.05$) was observed on the fecal moisture throughout the experiment.

DISCUSSION

In the current study, the inclusion of antibiotics did not affect the growth performance of pigs throughout the experiment, although it increased the dry matter digestibility and energy digestibility at the end of this study.

Table 4. Effects of bacterphage on fecal microflora and diarrhea score in growing pigs[1]

Items	NC	PC	BPT1	BPT2	SE[2]
Red Blood cell (10^6/μl)					
0 wk	6.23	6.20	6.53	6.64	0.17
6 wk	7.07	6.37	5.95	6.33	0.36
White blood cell (10^3/μl)					
0 wk	22.11	21.00	25.02	21.37	2.41
6 wk	21.75	20.65	20.70	19.40	1.95
Lymphocyte (%)					
0 wk	54.65	48.45	61.20	55.68	4.95
6 wk	60.68	54.10	59.88	63.91	4.76

[1] NC = Basal diet (negative control); PC = Basal diet+22 ppm tylosin (positive control); BPT1 = NC+0.025% bacterphage; BPT2 = NC+0.05% bacterphage. [2] Standard error. [3] Fecal score: 0, normal; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea.
[a,b] Means in the same row with different superscripts differ ($p<0.05$).

In agreement with this study, our previous studies (Wang et al., 2009; Yan et al., 2011b) also suggested pig fed the antibiotics supplemented diet did not affect the growth performance but led to a higher nutrient digestibility in growing pigs. As we known, the effect of antibiotic growth promoter (AGP) on the growth performance could be affected by many factors such as environment, animal age, and administration dosage. Therefore, we hypothesized that the reason for the lack effect of antibiotic could be attributed to the good sanitation condition of the experiment facility. It is well suggested that antibiotic supplementation could decrease the pathogenic bacterial in the intestine, and subsequently increased the nutrient digestibility of pigs. Our results suggested that the inclusion of antibiotics reduced the *E. coli* and *Salmonella* concentration compared with basal diet. Nagy and Fekete (1999) had previously suggested that *E. coli* are shed by clinically healthy animals, and may cause a drop in nutrient digestibility and growth performance in livestock. Forshell and Wierup (2006) also suggested that *Salmonella* is a pathogen of considerable importance in worldwide animal production. Therefore, the reason for the increase nutrient digestibility is likely to be the improved intestine gut health caused by antibiotics supplementation.

Similarly, administration of bacteriophge did not affect the growth performance compared with those without bacteriophage supplementation, which is partially in agreement with Gebru et al. (2010), who suggested that supplementation of bateriophgae did not affect the growth performance and feed intake in growing pigs under normal state. But interestingly, the inclusion of bacteriophage increased the nutrient digestibility compared with the basal diet, which was comparable to those with antibiotics supplementation. Barrow (2001) had previously reported that bacteriophages have therapeutic value as anti-*Salmonella* agent in pigs. Wall et al. (2010) also suggested that administration of anti-*Salmonella* phage to pigs challenged with *Salmonella* could reduce *Salmonella* colonization by 90 to 99.9% in the ileum and cecum. It is well known that gastrointestinal microflora play a number of important roles in animal production because intestine is the largest nutrient adsorption part in the pigs; therefore, a possible reason for the increased digestibility is likely to be the increased gut health of the pigs due to the bacteriopages supplementation. This hypothesis is strength by our results on the fecal microbial shedding, wherein the inclusion of bacteriophage decreased the *E. coli* and *Salmonella* concentration. Moreover, a higher *lactobacillus* concentration was also observed with the bacteriophages supplementation at the level of 0.5 g/kg. Metchnikoff (1908) had previously suggested that *lactobacillus* could balance the intestinal environment, prevent the growth of pathogenic bacteria and subsequently benefit the animal production. Therefore, the increased *lactobacillus*

Table 5. Effects of bacterphage on fecal microflora and diarrhea score in growing pigs[1]

Items	NC	PC	BPT1	BPT2	SE[2]
E. coli (log10 cfu/g)	6.55[a]	6.00[b]	6.32[ab]	6.14[b]	0.10
Lactobacillus (log10 cfu/g)	6.89[b]	6.93[b]	7.16[ab]	7.52[a]	0.14
Salmonella (log10 cfu/g)	3.62[a]	2.57[b]	2.21[b]	2.02[b]	0.51
Diarrhea score[3]	0.05	0.04	0.05	0.04	0.01
Fecal moisture	62.43	62.47	63.12	62.91	1.14

[1] NC = Basal diet (negative control); PC = Basal diet+22 ppm tylosin (positive control); BPT1 = NC+0.025% bacterphage; BPT2 = NC+0.05% bacterphage. [2] Standard error. [3] Fecal score: 0, normal; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea.
[a,b] Means in the same row with different superscripts differ ($p<0.05$).

concentration may also reflect the improved nutrient digestibility in the current study. The reason for improved *lactobacillus* is likely to be the reduced *E. coli* and *Salmonella* concentration, which provided a better ecosystem for the development of the *lactobacillus*.

It is well accepted that gastrointestinal is the largest immunologically competent organ in the body, the maturation and optimal development of the immune system depend on the development and composition of the indigenous microflora and vice versa (de Vrese and Marteau, 2007). Our results also indicated that the inclusion of antibiotics and bacteriophages benefited the microflora in the intestine. Therefore, we hypothesized that the blood characteristics could be affected by the inclusion of antibiotics or bacteriophages, because the blood characteristic is always considered as a sensitive response to various treatment. Unluckily, results of current study were out of anticipation and no difference was observed, which is inconsistent with our previous study (Yan et al., 2011c), who suggested that the inclusion of antibiotics could increase the lymphocyte concentration in growing pigs. It should be noted that the antibiotics used in this two studies were used in two different dosages, therefore, the reason for the difference may be the different dosages used in this study. Besides, to the best of our knowledge, there is no research investigating the effect of bacteriophages supplementation on the blood characteristics under normal states. Therefore, no comparison could be made with the current study. Collectively, we cannot make any statements regarding the effects of bacteriophages on the blood characteristics in the current study.

CONCLUSION

In conclusion, our study suggested that the bacteriophages at the level of 0.5 g/kg could be used as an antibiotics alternative for growing pigs.

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