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Author	Seonah Park1, Abdolreza Hosseindoust1, Junyoung Mun1, Sanghun Ha1, Priscilla Silvestre1, Sangsik Lee1, Elick Kinara1, Anushka Lokhande2, Youngin Kim3, and Jinsoo Kim1
Affiliation	1 Department of Animal Industry Convergence, Kangwon National University, Chucheon, Republic of Korea, 24341 2 Vansh Specialities, Mumbai, 400059, India 3 CTC Bio, Inc., Seoul, 138-858, Republic of Korea, 18569
ORCID (for more information, please visit https://orcid.org)	Seonah Park (https://orcid.org/0009-0007-6584-2777) Abdolreza Hosseindoust (https://orcid.org/0000-0001-9191-0613) Junyoung Mun (https://orcid.org/0000-0002-3075-7157) Sanghun Ha (https://orcid.org/0000-0003-3779-1144) Priscilla Silvestre (https://orcid.org/0009-0007-0473-2603) Sangsik Lee (https://orcid.org/0009-0003-2171-8388) Elick Kinara (https://orcid.org/0009-0003-1848-0399) Anushka Lokhande (https://orcid.org/0000-0003-1268-4932) Youngin Kim (https://orcid.org/0000-0001-6821-6084) Jinsoo Kim (https://orcid.org/0000-0002-9518-7917)
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Authors' contributions Please specify the authors' role using this form.	Conceptualization: Park SA, Kim JS. Data curation: Silvestre P, Kinara E. Formal analysis: Park SA, Lee SS, Mun JY, Kim YI. Methodology: Ha SH, Mun JY. Software: Silvestre P, Lee SS, Lokhande A. Validation: Hosseindoust A, Kim JS. Investigation: Park SA. Writing - original draft: Park SA, Hosseindoust A, Ha SH. Writing - review & editing: Park SA, Hosseindoust A, Ha SH, Mun JY, Silvestre P, Lee SS, Kinara E, Lokhande A, Kim YI, Kim JS.
Ethics approval and consent to participate	The animal care and experimental protocols used in the present study were approved by the Institution of Animal Care and Use Committee, Kangwon National University. (Ethical code: KW-241205-1).

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5 **CORRESPONDING AUTHOR CONTACT INFORMATION**

For the corresponding author (responsible for correspondence, proofreading, and reprints)	**Fill in information in each box below**
First name, middle initial, last name	Jinsoo Kim
Email address – this is where your proofs will be sent	kjs896@kangwon.ac.kr
Secondary Email address	
Address	Department of Animal Industry Convergence, Kangwon National University, Chucheon, Republic of Korea, 24341
Cell phone number	+82-10-2566-5961
Office phone number	+82-33-250-8614
Fax number	+82-33-259-5572

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Abstract

This study investigated whether the functional efficacy of probiotics (PRO), bacteriophages (BAC), and a phytogenic–β-glucan multi-component blend (COK) differs depending on the timing of administration relative to in-feed antibiotic (ANT) exposure in weaned pigs. A total of 120 piglets were assigned to five treatments: CON (basal diet), ANT (d 0-7 supplementation antibiotic), ANP (d 0-7 supplementation antibiotic + d 8-21 supplementation probiotics), ANB (d 0-7 supplementation antibiotic + d 8-21 supplementation bacteriophage), ANC (d 0-7 supplementation antibiotic + d 8-21 supplementation of multi-component blend). Growth performance, fecal consistency, nutrient digestibility, blood hormones, inflammatory cytokines, and hair cortisol were evaluated over a 21-day period. No additive exerted measurable effects during the antibiotic administration period (phase 1), indicating that amoxicillin masked functional responses during early post-weaning. Pigs receiving ANP and ANC exhibited higher overall average daily gain and average daily feed intake than CON ($p<0.05$). The ANC group showed greater crude protein digestibility than CON and ANT during phase 3 ($p<0.05$). ANP, ANB, and ANC supplementation increased serum IGF-1 than CON at d 21 ($p<0.05$). Both ANP and ANC tended to attenuate inflammatory cytokines in phase 3, whereas ANB supplementation produced minimal effects ($p=0.051$). Fecal score and hair cortisol were unaffected across treatments ($p>0.05$). These results demonstrate that functional additives provide limited benefit when co-administered with antibiotics but become effective once antibiotics are withdrawn, highlighting the importance of timing when integrating additives into antibiotic-restricted weaning programs.

Keywords: Weaned pigs, antibiotics, bacteriophage, phytogenic additives, supplementation timing.

INTRODUCTION

The weaning transition is a critical period in swine production characterized by abrupt dietary change, environmental stress, and immune underdevelopment, all of which predispose piglets to post-weaning diarrhea (PWD), impaired growth, and intestinal dysbiosis [1-3]. For decades, in-feed antibiotics such as amoxicillin have been widely applied to mitigate PWD and improve performance in weaned pigs [4]. However, concerns regarding antimicrobial resistance (AMR) and regulatory restrictions have led many countries to limit or ban the prophylactic use of antibiotics in livestock production [5]. Consequently, the swine industry has placed increasing emphasis on developing effective non-antibiotic strategies that can maintain gut health and growth without relying on conventional antimicrobials.

Various feed additives including probiotics, bacteriophages, organic acids, and phytogenic compounds have shown potential to modulate the gut microbiota, enhance intestinal integrity, and reduce PWD [6,7]. Probiotics such as *Bacillus subtilis*, *Enterococcus faecium*, and yeast strains contribute to pathogen inhibition and immune modulation, while bacteriophages selectively target harmful bacteria and may serve as precise antimicrobial tools [8]. Phytogenic mixtures containing essential oils, curcumin derivatives, or β-glucans have also been reported to exert anti-inflammatory, antioxidant, and antimicrobial effects in pigs [9]. Although these alternatives have shown promising outcomes, most studies have evaluated them either as complete antibiotic substitutes or as supplements after antibiotic withdrawal, with minimal attention given to how their efficacy varies depending on the timing of administration relative to antibiotic use.

Antibiotic exposure in young pigs is well-documented to disrupt gut microbial diversity, promote overgrowth of opportunistic pathogens, and markedly increase the abundance of antimicrobial-resistance genes (ARGs) within the intestinal ecosystem [10,11]. Recent metagenomic and metabolomic evidence further indicates that antibiotic-induced dysbiosis compromises intestinal barrier integrity, alters short-chain fatty acid production, and delays microbiome recovery long after antibiotic withdrawal [12,13]. Moreover, such disturbances compromise colonization resistance, elevate enteric pathogen risk, and may result in prolonged inflammatory responses and reduced nutrient absorption [14]. Therefore, the functional response to feed additives may differ substantially when they are provided together with antibiotics, during the microbial re-establishment period after antibiotic cessation, or in antibiotic-free conditions. Understanding the optimal window for additive supplementation is essential for establishing effective antibiotic-reduction programs without compromising animal health or productivity.

Accordingly, the present study evaluated the effects of probiotics, bacteriophages, and a multi-component blend when administered either during the amoxicillin-feeding period or after antibiotic withdrawal, allowing us to assess how the timing of additive supplementation influences their functional

efficacy. Growth performance, diarrhea incidence, inflammatory cytokines, and fecal microbiota were analyzed to determine whether these additives can support gut health under antibiotic exposure and during the subsequent recovery phase. This study provides practical evidence for developing optimized additive programs that align with antibiotic-restricted swine production systems.

MATERIAL AND METHODS

The Institutional Animal Care and Use Committee of Kangwon National University approved the animal care and experimental techniques utilized in this study (Ethical code: KW-241205-1).

Additive information

The feed additives used in this experiment were sourced from commercial suppliers. The antibiotic product containing amoxicillin (100 mg/kg) was obtained from CTC Bio, Inc. (Seoul, Republic of Korea). The probiotic product (CTC Bio, Inc., Seoul, Republic of Korea) contained a mixed culture of *Bacillus subtilis*, *Saccharomyces cerevisiae*, and *Enterococcus faecium*, with each strain guaranteed at 1.0×10^{10}CFU/g. The bacteriophage product (CTC Bio, Inc., Seoul, Republic of Korea) consisted of bacteriophages against *E. coli* (K88, K99, and F41), salmonella (*S. typhimurium* and *S. enteritidis*), and *Clostridium perfringens* (*C. perfringens* types A and C) with a minimum activity of 1.0×10^{11} CFU/g. The multi-component blend (Vansh Specialities, Mumbai, India) contained β-(1,3;1,6)-glucan, bacteriophage (CTC Bio, Inc., Seoul, Republic of Korea), lysozyme, bile extract, curcumin extract, thymol, and oregano.

Animals, experimental designs, and procedures

The study was conducted on a commercial farm in Haman, Gyeongsangnam-do, Republic of Korea. A total of 120 weaning pigs (Landrace × Yorkshire × Duroc, initial body weight (BW) 4.70 ± 0.01 kg) were randomly assigned to 5 treatments. All treatments consisted of 6 replicates with 4 pigs per pen. The experiment was conducted for 21 days (phase 1, d 0-7; phase 2, d 8-14; phase 3, d 15-21). During phase 1, pigs were assigned to either a control or antibiotic feeding program. During phase 2, pigs were allocated to one of the following dietary treatments; CON (basal diet), ANT (d 0-7 supplementation antibiotic), ANP (d 0-7 supplementation antibiotic + d 8-21 supplementation probiotics), ANB (d 0-7 supplementation antibiotic + d 8-21 supplementation bacteriophage), ANC (d 0-7 supplementation antibiotic + d 8-21 supplementation of multi-component blend). All pigs received zinc oxide supplementation from 0 to 14, following standard weaning management practices. The experimental diet mixing ratio and chemical composition used in this study are presented in Table 1, and all nutrient levels were formulated to meet or exceed the nutrient requirements recommended by the NRC [15].

Feed was provided in mash form, and both feed and water were offered ad libitum throughout the trial. Pigs were housed in environmentally controlled weaning pens equipped with plastic slatted floors. Housing, feeding management, sanitation, and biosecurity procedures followed the farm's standard operating protocols.

Experimental procedures and sample collection

The experimental pig's BW was measured at the beginning and end of every period for average daily gain (ADG) calculation. The amount of feed offered and feed remaining in the feeders were recorded daily and at the end of each phase to calculate average daily feed intake (ADFI). Feed efficiency (G:F) was calculated based on ADG and ADFI. Mortality was 0%, and no pigs were removed during the experimental period. Each treatment consisted of 6 replicate pens with 4 pigs per pen, which were maintained until the end of the study.

Fecal score

Fecal consistency was evaluated on d 1-7, 14, and 21 of the experimental periods. Fecal score was assessed visually by trained personnel using a five-point scoring system, where 1 = hard and dry feces, 2 = normal firm feces, 3 = soft and formed feces, 4 = mild diarrhea (soft and unformed), and 5 = severe watery diarrhea. Fecal score was recorded at the pen level, and the mean score of each pen was used as the experimental unit for statistical analysis.

Nutrient digestibility

Chromic oxide (Cr_2O_3, 0.25%) was incorporated into all experimental diets as an indigestible marker 7 days prior to fecal collection. On d 7 and d 21, fecal samples were collected from one randomly selected pig per pen via gentle rectal massage to ensure representative sampling across replicates. Feed samples and the corresponding fecal samples were pooled within each pen and dried in a forced-air oven at 60°C for 72 h. Dried samples were subsequently ground through a 1-mm screen using a Thomas Model 4 Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA). Ground samples were analyzed in triplicate for dry matter (DM) using AOAC (2007) method 930.15, crude protein (CP) using AOAC (2007) method 990.03, and ether extract (EE) using AOAC (2007) method 960.39. Chromium concentrations in feed and feces were determined using an automated spectrophotometer (Jasco V-650, Jasco Corp., Tokyo, Japan). Apparent nutrient digestibility was calculated using the indicator method as follows:

Digestibility (%) = 100 − [100 × (marker in feed / marker in feces) × (nutrient in feces / nutrient in feed)].

Blood sampling and serum preparation

Blood samples were collected from one representative pig per pen (n = 6 per treatment) on d 7 and d 21 of the experimental period. Approximately 5 mL of blood was obtained from the jugular vein using sterile vacuum tubes without anticoagulant. Samples were allowed to clot at room temperature for 30 min and centrifuged at 3,000 × g for 15 min at 4°C to obtain serum. The separated serum was transferred

into sterile microtubes and stored at −80°C until analysis.

Blood Hormone Analysis

Serum growth hormone (GH, MBS264732) and insulin-like growth factor-1 (IGF-1, MBS2503268) concentrations were analyzed using porcine-specific ELISA kits (MyBioSource, San Diego, CA, USA). All procedures were performed according to the manufacturer's instructions, and absorbance values were measured using a microplate reader (BioTek Instruments, Winooski, VT, USA).

Inflammatory cytokine analysis

Serum concentrations of tumor necrosis factor-α (TNF-α, MBS2019932), interleukin-1β (IL-1β, MBS2021728), interleukin-10 (IL-10, MBS761474), and interleukin-2 (IL-2, MBS453421) were determined using porcine-specific ELISA kits (MyBioSource, San Diego, CA, USA), following the manufacturer's protocols. Absorbance readings were obtained with the same microplate reader used for hormone assays.

Stress marker

Hair cortisol concentration was determined according to the method described by Tajudeen et al. [16], with minor modifications. Hair samples were collected from one representative pig per pen (n = 6 per treatment) on d 7 and d 21 of the experimental period. To ensure consistent regrowth, a small patch of hair from the dorsal neck region was shaved at the beginning of the trial. Newly grown hair (approximately 20–30 mg) was clipped as close to the skin surface as possible and stored in sterile tubes at room temperature. Hair samples were washed three times with isopropanol to remove external contaminants and then dried in a vacuum dryer at 35°C. The dried samples were placed into an EML plastic tube containing steel beads and pulverized using a bead beater (taco™Prep, GeneReach, Taichung, Taiwan) until finely powdered. For cortisol extraction, approximately 10 mg of powdered hair was incubated in 1.0 mL of methanol for 24 h at room temperature with continuous agitation. After incubation, samples were centrifuged at 3,000 × g for 10 min, and the supernatant was transferred to a clean tube. Methanol extracts were evaporated under nitrogen gas and reconstituted using the assay buffer supplied in the ELISA kit. Hair cortisol concentration was quantified using a cortisol ELISA kit (ADI-900-071, Enzo Life Sciences, Farmingdale, NY, USA), following the manufacturer's instructions. Absorbance was measured using a microplate reader (BioTek Instruments, Winooski, VT, USA), and cortisol levels were expressed as pg/mg of hair.

Fecal microbiota

Fecal samples were immediately snap-frozen in liquid nitrogen after collection, transported to the laboratory under frozen conditions, and subsequently stored at –80 °C until further processing. Genomic DNA was isolated from the samples using the QIAamp Fast DNA Stool Mini Kit (Cat. No. 51604/2016), following the manufacturer's protocol for stool-derived material. Quantification of microbial populations was conducted using real-time quantitative PCR (qPCR). The assay targeted *Lactobacillus spp.*, *Bifidobacterium spp.*, *Clostridium spp.*, *Salmonella spp.*, *Escherichia coli*, and β-actin, which

served as the reference gene. Primer sequences used for amplification are listed in Table 2. qPCR amplifications were performed for 40 cycles, starting with a 15-second activation/denaturation step at 95 °C. Fluorescence derived from SYBR Green incorporation was monitored at the 72 °C extension phase. Microbial counts were calculated based on Ct values obtained from ten-fold diluted DNA templates. All reactions were carried out using the Rotor-Gene QIAGEN 2plex platform (Serial No. 0312272, Corvette Research).

Statistical analysis

All data were analyzed using the General Linear Model (GLM) procedure of SAS (Version 9.4; SAS Institute Inc., Cary, NC, USA). Treatment was considered as the sole fixed effect because the experimental design was based on a single-factor arrangement of dietary treatments. The pen (4 pigs per pen) was used as the experimental unit for growth performance and blood-related parameters, whereas the individual pig served as the experimental unit for nutrient digestibility, fecal score, and microbiota analysis. When a significant overall treatment effect was detected, mean separation was performed using Tukey's honest significant difference (HSD) test. All results are presented as means ± standard error of the mean (SEM). Statistical significance was declared at $p < 0.05$, while values between $0.05 \leq p < 0.10$ were considered to indicate a tendency.

RESULTS

Growth performance

Final BW was significantly higher in pigs fed the ANP and ANC diet compared with the CON group ($p = 0.021$; Table 3). During phase 1, 2, and 3, no differences were observed among treatments in ADG, ADFI, or G:F ($p>0.05$). For the overall period, pigs fed the ANP and ANC diets showed greater ADG ($p = 0.032$) and ADFI ($p=0.017$) than the CON treatment, whereas G:F remained unaffected by dietary treatment.

Fecal score

Fecal consistency progressively improved with advancing age in all treatment groups (Figure 1). However, no significant differences were detected among treatments throughout the entire experimental period.

Nutrient digestibility

During phase 1, no significant differences were observed among treatments in DM, CP, or EE digestibility (Table 4). In phase 3, CP digestibility was significantly higher ($p=0.022$) in pigs fed the ANC diet compared with the CON and ANT treatment, while DM and EE digestibility remained unaffected across treatments.

Blood hormone

During phase 1, no significant differences were observed among treatments in serum GH or IGF-1 concentrations (Table 5). In phase 3, pigs the ANP, ANB, and ANC group showed the higher values compared with the CON treatment ($p<0.001$). However, GH concentration did not differ among treatments.

Inflammation cytokine

During phase 1, no significant differences were detected among treatments for all measured cytokines, including TNF-α, IL-1β, IL-10, and IL-2 (Table 6). In phase 3, TNF-α was significantly reduced when pigs fed ANP compared with CON ($p=0.037$), while IL-1β showed a tendency to differ among treatments ($p=0.051$). In contrast, IL-10 and IL-2 concentrations remained unaffected by dietary treatments throughout the experimental period.

Stress marker

Hair cortisol concentrations did not significantly differ among treatments throughout the entire experimental period (Table 7).

Fecal microbiota

During phase 1, the fecal microbiota was not influenced by the use of antibiotics (Table 8). *Lactobacillus* was higher in the ANP and ANC groups than in the CON group ($p = 0.001$). *Clostridium* was significantly lower in ANP and APC compared with CON, ANC, and ANB ($p = 0.012$). *Escherichia coli* was reduced in ANP and ANC relative to CON and ANT ($p = 0.002$).

DISCUSSION

The absence of treatment differences during the first 7 days post-weaning indicates that amoxicillin exposure largely overshadowed the potential effects of the supplemented additives. This outcome is not unexpected, as antibiotic treatment is known to markedly suppress microbial activity, reduce luminal antigenic load, and attenuate the metabolic pathways through which functional feed additives typically exert their effects [17,18]. Consequently, the early post-weaning period in this experiment represented an environment in which probiotics, bacteriophages, and phytogenic compounds had limited opportunity to influence gut physiology.

Clear treatment effects emerged only after antibiotic withdrawal, where pigs receiving ANP and ANC exhibited improved growth performance compared with the CON group. These findings suggest that both additives contributed to the restoration of intestinal function during the microbial re-establishment period. Probiotics are well documented to support epithelial repair, enhance nutrient transporter expression, and stabilize the microbial community following perturbation [19,20]. The elevated IGF-1

concentrations observed in ANP pigs during phase 3 further support improved nutrient assimilation and mucosal integrity, as IGF-1 is closely associated with anabolic recovery and reduced intestinal inflammation [21]. Together, these results indicate that probiotic supplementation became physiologically relevant once the suppressive effects of amoxicillin dissipated.

Similarly, pigs fed ANC showed enhanced CP digestibility and improved overall ADG and ADFI. The multi-component composition of the COK additive including β-glucan, *Bacillus subtilis*, lysozyme, and plant-derived bioactive compounds is known to support intestinal recovery by enhancing digestive secretions, improving oxidative balance, and promoting brush-border enzyme activity during post-weaning restoration [22,23]. The improved protein digestibility observed in this group is therefore consistent with the combined functional effects of these components. The delayed emergence of the response also corresponds with previous observations that the benefits of plant-derived functional additives tend to manifest most clearly when epithelial regeneration and microbial recolonization are underway [24].

In contrast, ANB yielded performance and physiological responses comparable to CON and ANT, suggesting a largely neutral effect under the conditions of this experiment. This outcome is not contradictory to existing knowledge: bacteriophages require sufficient densities of susceptible host bacteria to propagate effectively, and antibiotic administration during phase 1 likely reduced host abundance below the threshold necessary for meaningful phage activity [25,26]. The neutral response observed here therefore reflects environmental constraints rather than phage inefficacy and is consistent with studies showing that phage benefits are context-dependent and typically emerge under defined pathogen challenge [27].

Despite observed improvements in growth performance and digestive parameters, cortisol concentrations remained unchanged across treatments. This suggests that the observed benefits were not mediated through systemic stress reduction, at least as measured by hair cortisol. Hair cortisol reflects long-term HPA-axis activity but is influenced by multiple external factors, including environmental conditions, hair growth rate, and sampling variability [28-30]. Therefore, the lack of cortisol response does not necessarily contradict improvements in gut health or immune status, but rather indicates that these effects may occur independently of measurable changes in systemic stress biomarkers.

Collectively, results from Experiment 1 indicate that antibiotic administration during the immediate post-weaning period suppresses the functional activity of multiple classes of feed additives. However, once antibiotic pressure was removed, probiotics and phytogenic compounds supported intestinal recovery, leading to improved nutrient utilization, growth performance, and inflammatory status. The findings highlight the importance of timing, suggesting that feed additives may be most effective when

targeted to the post-antibiotic recovery phase rather than co-administered with antibiotics. This has practical implications for farms that continue to rely on early-life antimicrobial meta phylaxis, as strategic positioning of additives could enhance overall health outcomes without altering antibiotic protocols.

CONCLUSION

After antibiotic withdrawal, probiotics and the multi-component blend improved overall ADG and ADFI, and multi-component blend supplementation after antibiotics additionally enhanced CP digestibility during the late nursery period. Probiotics supplementation after antibiotic use elevated serum IGF-1, and both probiotics and multi-component blend reduced inflammatory cytokines, whereas bacteriophages produced minimal effects. These findings indicate that the use of probiotics or multi-component blend were effective when supplied after antibiotics are discontinued.

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Tables

Table 1. Formula and chemical composition of experimental basal diet (as-fed basis)

Day	0-14	15-21
Crude protein (CP) level, %	20	18
Feed ingredients, %		
Corn	41.66	50.21
Soybean meal_45%	14.84	17.91
Whey	11.00	10.00
Lactose	9.00	6.00
Fishmeal_70%	5.00	3.00
Spray dried plasma protein	5.00	2.00
Animal fat	4.01	3.24
Sugar	6.00	4.00
$_{L}$-Lysine_78.8%	0.36	0.44
$_{DL}$-Methionine_98%	0.20	0.20
$_{L}$-Threonine_99%	0.09	0.14
$_{L}$-Tryptophan_10%	0.14	0.27
Limestone	1.00	1.01
Mono calcium phosphate	0.47	0.65
Salt	0.50	0.50
Choline chloride_50%	0.05	0.05
Zinc oxide	0.3	-
Vitamin premix[1]	0.11	0.11
Mineral premix[2]	0.22	0.22
Phytase	0.05	0.05
Total	100.00	100.00
Calculated composition, %		
Metabolizable energy, kcal/kg	3,400	3,350
Net energy, kcal/kg	2,548	2,487
CP	20.00	18.00
Crude fat	6.01	5.25
Calcium	0.80	0.70
Available phosphorus	0.40	0.33
SID Lysine	1.35	1.23
SID Methionine	0.47	0.44
SID Methionine+Cysteine	0.74	0.68
SID Threonine	0.79	0.73
SID Tryptophan	0.22	0.20

STTP, ; SID, standardized ileal digestibility. [1]Supplied per kilogram of vitamin premix: 3,000 IU vitamin A, 300 IU vitamin D_3, 50 IU vitamin E, 2 mg vitamin K_3, 2 mg vitamin B_1, 5 mg vitamin B_2, 10 mg vitamin B_6, 25 mg vitamin B_{12}, 15 mg pantothenic acid, 30 mg niacin, 0.1 mg biotin, 0.3 mg folic acid.

[2]Supplied per kilogram of mineral premix: 100 mg Fe, 6.00 mg Cu, 4 mg Mn, 100 mg Zn, 0.14 mg I, 0.3 mg Se.

Table 2. Primer information.

Item	Primer sequence	Reference
Lactobacillus spp.	F: AGCAGTAGGGAATCTTCCA R: CACCGCTACACATGGAG	Walter et al., 2001
Bifidobacterium spp.	F: TCGCGTCYGGTGTGAAAG R: CCACATCCAGCRTCCAC	Rinttila et al., 2004
Clostridium spp.	F: AAAGGAAGATTAATACCGCATAA R: ATCTTGCGACCGTACTCCCC	Mirhosseini et al., 2010
Salmonella spp.	F: CCT ACG GGA GGC AGC AG R: CCG TCA ATT CCT TTR AGT TT	Halatsi et al., 2006
Escherichia coli	F: CGAGGGCTTGATGTCTATCAG R: TCAGTATAACGGCCACAGTCC	Park et al., 2006
β-Actin	F: CTCCTTCTTGGGCATGGA R: CGCACTTCATGATCGAGTTGA	Tajudeen et al., 2024

Table 3. Effects of dietary antibiotics and feed additives supplementation on growth performance of weaning pigs.

Item[1]	CON	ANT	ANP	ANB	ANC	SEM	p-value
Antibiotic (Day 0-7)	-	+	+	+	+		
Additives (Day 8-21)	-	-	probiotics	bacteriophage	COK		
BW, kg							
Initial	4.70	4.71	4.71	4.71	4.70	0.01	0.687
Final	9.34^{c}	9.48^{bc}	9.81^{a}	9.70^{ab}	9.74^{a}	0.13	0.021
Phase 1 (d 0-7)							
ADG, g/d	187	195	199	199	198	11.50	0.811
ADFI, g/d	253	263	269	266	269	15.44	0.821
G:F	0.738	0.743	0.739	0.746	0.736	0.01	0.541
Phase 2 (d 8-14)							
ADG, g/d	219	223	252	241	240	23.07	0.612
ADFI, g/d	301	304	343	328	324	30.40	0.632
G:F	0.728	0.735	0.736	0.735	0.739	0.01	0.653
Phase 3 (d 15-21)							
ADG, g/d	256	262	277	272	282	26.97	0.870
ADFI, g/d	357	367	385	377	395	37.33	0.869
G:F	0.717	0.714	0.719	0.721	0.713	0.01	0.676
Overall (d 0-21)							
ADG, g/d	220^{c}	227^{bc}	243^{a}	237^{ab}	240^{a}	8.01	0.032
ADFI, g/d	304^{c}	311^{bc}	332^{a}	324^{ab}	329^{a}	9.38	0.017
G:F	0.728	0.731	0.731	0.734	0.730	0.01	0.336

[1]CON, basal diet only (d 0-21); ANT, basal diet+0.17% antibiotic (d 0-7), and basal diet (d 8-21); ANP, basal diet+0.17% antibiotic (d 0-7), and basal diet+0.05% probiotics (d 8-21); ANB, basal diet+0.17% antibiotic (d 0-7), and basal diet+0.01% bacteriophage (d 8-21); ANC, basal diet+0.17% antibiotic (d 0-7),and basal diet+0.075% multi-component blend (COK) (d 8-21).
All treatments were supplemented with 0.25% zinc oxide from d 0 to 14.
SEM, standard error of means; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, feed efficiency.
[ab]Mean values within a row with unlike superscript letters were significantly different (P<0.05).

Table 4. Effects of dietary antibiotics and feed additives supplementation on nutrient digestibility of weaning pigs.

Item[1]	CON	ANT	ANP	ANB	ANC	SEM	p-value
Antibiotic (Day 0-7)	-	+	+	+	+		
Additives (Day 8-21)	-	-	probiotics	bacteriophage	COK		
Phase 1 (d 7)							
DM, %	85.71	86.71	86.30	86.68	86.06	0.64	0.487
CP, %	79.49	80.95	81.16	80.47	81.48	1.99	0.873
EE, %	70.24	71.75	70.59	71.66	71.70	1.03	0.434
Phase 3 (d 21)							
DM, %	87.65	88.22	89.52	88.95	89.53	1.33	0.558
CP, %	86.04[b]	86.52[b]	88.81[ab]	87.99[ab]	89.20[a]	1.06	0.022
EE, %	76.20	76.90	77.63	77.16	79.30	1.53	0.352

[1]CON, basal diet only (d 0-21); ANT, basal diet+0.17% antibiotic (d 0-7), and basal diet (d 8-21); ANP, basal diet+0.17% antibiotic (d 0-7), and basal diet+0.05% probiotics (d 8-21); ANB, basal diet+0.17% antibiotic (d 0-7), and basal diet+0.01% bacteriophage (d 8-21); ANC, basal diet+0.17% antibiotic (d 0-7),and basal diet+0.075% multi-component blend (COK) (d 8-21).
All treatments were supplemented with 0.25% zinc oxide from d 0 to 14.
SEM, standard error of means; DM, dry matter; CP, crude protein; EE, ether extract.
[ab] Mean values within a row with unlike superscript letters were significantly different ($P<0.05$).

Table 5. Effects of dietary antibiotics and feed additives supplementation on blood hormone of weaning pigs

Item[1]	CON	ANT	ANP	ANB	ANC	SEM	p-value
Antibiotic (Day 0-7)	-	+	+	+	+		
Additives (Day 8-21)	-	-	probiotics	bacteriophage	COK		
Phase 1 (d 7)							
GH, ng/ml	1.75	1.95	2.17	2.12	2.14	0.18	0.153
IGF-1, ng/ml	1.87	1.91	1.94	1.92	1.94	0.13	0.981
Phase 3 (d 21)							
GH, ng/ml	2.29	2.42	2.69	2.53	2.69	0.22	0.326
IGF-1, ng/ml	2.69[b]	3.02[ab]	3.57[a]	3.43[a]	3.47[a]	0.20	<0.001

[1]CON, basal diet only (d 0-21); ANT, basal diet+0.17% antibiotic (d 0-7), and basal diet (d 8-21); ANP, basal diet+0.17% antibiotic (d 0-7), and basal diet+0.05% probiotics (d 8-21); ANB, basal diet+0.17% antibiotic (d 0-7), and basal diet+0.01% bacteriophage (d 8-21); ANC, basal diet+0.17% antibiotic (d 0-7),and basal diet+0.075% multi-component blend (COK) (d 8-21).

All treatments were supplemented with 0.25% zinc oxide from d 0 to 14.

SEM, standard error of means; GH, growth hormone; IGF-1, insulin-like growth factor-1.

[ab] Mean values within a row with unlike superscript letters were significantly different ($P<0.05$).

Table 6. Effects of dietary antibiotics and feed additives supplementation on inflammation cytokine of weaning pigs

Item[1]	CON	ANT	ANP	ANB	ANC	SEM	p-value
Antibiotic (Day 0-7)	-	+	+	+	+		
Additives (Day 8-21)	-	-	probiotics	bacteriophage	COK		
Phase 1 (d 7)							
TNF-α, pg/ml	55.84	46.07	44.90	43.92	47.68	5.20	0.114
IL-1β, pg/ml	40.96	37.30	36.27	37.17	36.94	8.97	0.986
IL-10, pg/ml	31.19	42.34	40.90	42.77	41.77	9.31	0.704
IL-2, pg/ml	4.72	4.28	4.70	4.34	4.52	0.64	0.950
Phase 3 (d 21)							
TNF-α, pg/ml	32.13[a]	27.18[ab]	21.09[b]	23.55[ab]	22.13[ab]	4.04	0.037
IL-1β, pg/ml	27.06	23.91	17.29	20.50	19.39	3.31	0.051
IL-10, pg/ml	28.27	32.64	30.94	32.18	31.48	4.69	0.897
IL-2, pg/ml	2.76	2.88	3.10	2.93	2.99	0.49	0.968

[1]CON, basal diet only (d 0-21); ANT, basal diet+0.17% antibiotic (d 0-7), and basal diet (d 8-21); ANP, basal diet+0.17% antibiotic (d 0-7), and basal diet+0.05% probiotics (d 8-21); ANB, basal diet+0.17% antibiotic (d 0-7), and basal diet+0.01% bacteriophage (d 8-21); ANC, basal diet+0.17% antibiotic (d 0-7),and basal diet+0.075% multi-component blend (COK) (d 8-21).
All treatments were supplemented with 0.25% zinc oxide from d 0 to 14.
SEM, standard error of means; TNF-α, tumor necrosis factor-alpha; IL-1β, interleukin-1beta; IL-10, interleukin-10; IL-2, interleukin-2.
[ab] Mean values within a row with unlike superscript letters were significantly different (P<0.05).

Table 7. Effects of dietary antibiotics and feed additives supplementation on stress marker of weaning pigs

Item[1]	CON	ANT	ANP	ANB	ANC	SEM	p-value
Antibiotic (Day 0-7)	-	+	+	+	+		
Additives (Day 8-21)	-	-	probiotics	bacteriophage	COK		
Phase 1 (d 7)							
Cortisol, pg/ml	144.01	134.07	132.90	132.08	132.29	9.58	0.696
Phase 3 (d 21)							
Cortisol, pg/ml	101.30	97.18	89.09	93.55	92.13	6.74	0.437

[1]CON, basal diet only (d 0-21); ANT, basal diet+0.17% antibiotic (d 0-7), and basal diet (d 8-21); ANP, basal diet+0.17% antibiotic (d 0-7), and basal diet+0.05% probiotics (d 8-21); ANB, basal diet+0.17% antibiotic (d 0-7), and basal diet+0.01% bacteriophage (d 8-21); ANC, basal diet+0.17% antibiotic (d 0-7),and basal diet+0.075% multi-component blend (COK) (d 8-21).
All treatments were supplemented with 0.25% zinc oxide from d 0 to 14.
SEM, standard error of means.

Table 8. Effects of dietary antibiotics and feed additives supplementation on fecal microbiota of weaning pigs

Item[1]	CON	ANT	ANP	ANB	ANC	SEM	p-value
Antibiotic (Day 0-7)	-	+	+	+	+		
Additives (Day 8-21)	-	-	probiotics	pacteriophage	COK		
Phase 1 (d 7)							
Lactobacillus	1.00	1.10	1.08	1.04	1.09	0.05	0.432
Bifidobacterium	1.00	1.05	1.06	1.10	1.07	0.06	0.551
Clostridium	1.00	1.02	0.99	0.96	0.98	0.05	0.421
Escherichia coli	1.00	0.97	0.96	0.97	0.95	0.06	0.389
Phase 3 (d 21)							
Lactobacillus	1.00^{ab}	0.97^{b}	1.21^{a}	1.11^{ab}	1.19^{a}	0.05	0.001
Bifidobacterium	1.00	1.02	1.07	1.06	1.08	0.06	0.633
Clostridium	1.00^{a}	0.97^{ab}	0.92^{ab}	0.99^{ab}	0.91^{b}	0.04	0.012
Escherichia coli	1.00^{a}	0.96^{ab}	0.89^{ab}	0.91^{ab}	0.85^{b}	0.05	0.002

[1]CON, basal diet only (d 0-21); ANT, basal diet+0.17% antibiotic (d 0-7), and basal diet (d 8-21); ANP, basal diet+0.17% antibiotic (d 0-7), and basal diet+0.05% probiotics (d 8-21); ANB, basal diet+0.17% antibiotic (d 0-7), and basal diet+0.01% bacteriophage (d 8-21); ANC, basal diet+0.17% antibiotic (d 0-7),and basal diet+0.075% multi-component blend (COK) (d 8-21).

All treatments were supplemented with 0.25% zinc oxide from d 0 to 14.

SEM, standard error of means; DM, dry matter; CP, crude protein; EE, ether extract.

[ab] Mean values within a row with unlike superscript letters were significantly different (P<0.05).

Figure legends

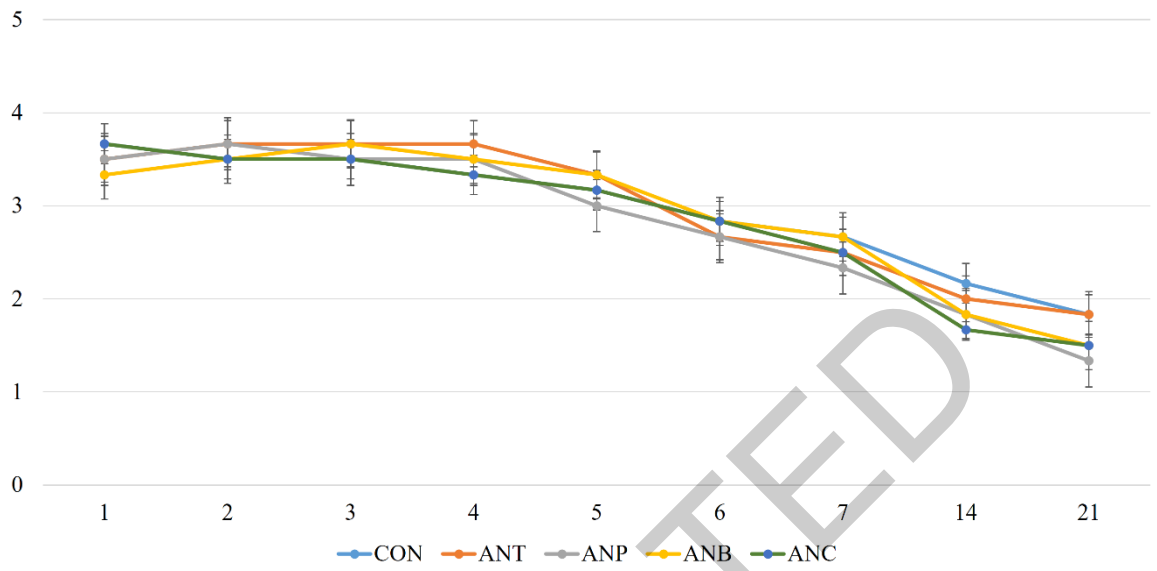

Figure 1. Effects of dietary antibiotics and feed additives supplementation on fecal score of weaning pigs. *Mean values within a row with unlike superscript letters were significantly different ($p<0.05$), and † indicates a tendency ($0.05 \leq p < 0.10$). Fecal consistency was scored using a 5-point scale, where 1 = hard and dry (constipated), 2 = firm and well-formed, 3 = soft and formed, 4 = very soft and unformed (pasty), and 5 = watery diarrhea. Higher scores indicate looser feces and greater severity of diarrhea. [1]CON, basal diet only (d 0-21); ANT, basal diet+0.17% antibiotic (d 0-7), and basal diet (d 8-21); ANP, basal diet+0.17% antibiotic (d 0-7), and basal diet+0.05% probiotics (d 8-21); ANB, basal diet+0.17% antibiotic (d 0-7), and basal diet+0.01% bacteriophage (d 8-21); ANC, basal diet+0.17% antibiotic (d 0-7),and basal diet+0.075% multi-component blend (COK) (d 8-21).