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# Gut microbiota alteration with growth performance, histopathological lesions, and immune responses in *Salmonella* Typhimurium-infected weaned piglets

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ABSTRACT

*Salmonella* Typhimurium can cause gastroenteritis in weaned piglets, which are particularly vulnerable to dietary changes and dysfunction of their immature organs. The colonization of *S.* Typhimurium could disrupt the gut microbiota and increase susceptibility to the bacterium. This study aimed to investigate the alterations of gut microbiota in *S.* Typhimurium-infected weaned piglets. Ten 49-day-old pigs were divided into two groups: *S.* Typhimurium-inoculated (ST, $n = 6$) and negative control (NC, $n = 4$) groups. The body weight and *S.* Typhimurium fecal shedding were monitored for 14 days after *S.* Typhimurium inoculation (dpi). The intestinal tissues were collected at 14 dpi; histopathological lesions and cytokine gene expression were evaluated. The gut microbiome composition and short-chain fatty acid concentrations were analyzed in fecal samples collected at 14 dpi. The average daily gain and gut microbiota alpha diversity in ST group tended to be lower than NC group at 14 dpi. Linear discriminant analysis effect size results showed a significant increase in the abundance of two genera and five species, while a significant decrease was observed in the five genera and nine species within the gut microbiota of ST group. Among the significantly less abundant bacteria in the ST group, *Lachnospira eligens* and *Anaerobium acetethylicum* produce acetate and butyrate, and may be considered as key *S.* Typhimurium infection-preventing bacteria. The overall results provide invaluable information about changes in the gut microbiota of *S.* Typhimurium-infected weaned piglets, which can be used to develop alternative measures to antibiotics and prevent ST bacterial infection.

## Introduction

Pigs are a substantial source of foodborne non-typhoidal *Salmonella* infections that cause health problems globally (Callaway et al., 2008). Among them, *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) is one of the most common serotypes in pigs that is responsible for diarrhea, gastroenteritis, and systemic infection (Badia et al., 2012; Oh et al., 2016). *S.* Typhimurium can infect pigs of all ages, but piglets in the post-weaning period are particularly susceptible due to sudden dietary changes and immature organ functions (López-Colom et al., 2019).

Successful colonization of *S.* Typhimurium is essential for the progression of infection, as it causes considerable changes in the gut environment. These changes lead to decreased disease resistance in pigs, making them more susceptible to bacterial infections (Kempf et al., 2023; Martins et al., 2013). In particular, *S.* Typhimurium infection in piglets destroys the immature gut barriers; causes inflammation, necrosis, and ulceration in the intestinal tract; and deteriorates the gut microbiome composition and environment (Badia et al., 2012; Oh et al.,

*Abbreviations:* ADG, average daily gain; AMR, antimicrobial resistance; ASV, amplicon sequence variants; IL, interleukin; LDA, linear discriminant analysis; LEfSe, linear discriminant analysis effect size; NC, negative control; PCoA, principal coordinate analysis; PCR, polymerase chain reaction; RT-PCR, reverse transcription polymerase reaction test; SCFA, short-chain fatty acid.

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2016). During *S.* Typhimurium infection, certain cytokines, such as interleukin (IL)-17A and IL-18, are essential for eliminating bacteria and regulating inflammatory responses (Eckmann & Kagnoff, 2001; Müller et al., 2016). The maintenance of a healthy gut microbiota could help protect against colonization by pathogenic microbes through various mechanisms. Protection measures include direct responses (such as toxin production and competition for nutrients and space) and indirect support by promoting the function of the intestinal barrier (Brosschot & Reynolds, 2018). However, only few studies have investigated the alterations of gut microbiota in *S.* Typhimurium-infected pigs, especially in weaned piglets (Barba-Vidal et al., 2017; Nair et al., 2019).

Traditionally, various antimicrobials have been used to treat *S.* Typhimurium infections in pigs. However, this practice can lead to increased antimicrobial resistance (AMR) of multiple bacteria in the gut of weaned piglets, including pathogenic bacteria (Jajere, 2019). In addition, the presence of antibiotic residues in the environment due to their widespread usage has become a major worldwide concern, particularly regarding antimicrobial abuse in livestock (Gresse et al., 2017; Jajere, 2019). Numerous previous studies suggested that altering the gut microbiota compositions by feeding probiotics and/or prebiotics could be a potential alternative to help mitigate the negative effects of *S.* Typhimurium infection in pigs (Argüello et al., 2018; Guevarra et al., 2019; O'Doherty et al., 2021; Pan et al., 2017; Wang et al., 2021).

The authors aimed to investigate the alterations of gut microbiota in *S.* Typhimurium-infected weaned piglets. The results of this study could offer insights into the composition of the gut microbiome of weaned piglets infected with *S.* Typhimurium, along with trends in bacterial shedding, histopathological lesions, and immune responses in intestinal tract organs. Additionally, the results may contribute to the further research in the development of alternative treatment methods to antibiotics for the prevention of *S.* Typhimurium infection at pig farms.

## Materials and methods

### *Ethics statement*

All animal experiments were approved by the Institutional Animal Care and Use Committee of the National Institute of Animal Science, Republic of Korea (approval number: NIAS 2021-503).

### *Experimental design*

Ten castrated 25-day-old male piglets (Landrace × Yorkshire) were purchased from the same herd on a commercial farm. The average weight of the piglets was 4.66 ± 0.61 kg. The piglets were randomly divided into *S.* Typhimurium-inoculated (ST, $n = 6$) and negative control (NC, $n = 4$) groups. All pigs were accommodated in the same experimental farm in two different pens. The distance between the two pens was approximately 3 m, and at disinfectant foot bath was placed in front of the entrance and each pen. All the experimental equipment and the approaches to each pen were kept strictly separated. All animals were monitored daily for 21 days by designated veterinarians before *S.* Typhimurium inoculation. The formulation of the diet was determined according to the nutritional requirements suggested by the Korean feeding standard for pigs (Table 1) (National Institute of Animal Science, 2017). The ST group pigs were orally inoculated with $1 \times 10^8$ colony-forming units of *S.* Typhimurium strain LT2 (ATCC 19585) at 49 days of age. The NC group pigs were treated as the mock infection group. All experimental pigs were euthanized and necropsied 14 days after *S.* Typhimurium inoculation (dpi). The jejunum, ileum, colon, and cecum tissue samples were immediately collected from each necropsied pig.

### *Sampling, bacterial isolation, and histopathology*

Fecal samples were collected at various time points (0, 2, 5, 8, and 11 dpi). Fecal and intestinal tissues were obtained from necropsied pigs at 14 dpi. Bacterial cultures and polymerase chain reaction (PCR) tests were performed as described previously (Yi et al., 2023). The collected tissue samples were preserved in 10 % neutral-buffered formalin and embedded in paraffin wax. Sections were prepared and stained according to our previous study (Yi et al., 2023). Histopathological lesions were evaluated using previously established criteria scored on a scale of 0–5, using the following parameters: no lesions = 0; villi shorten = 1; complete villi erosion = 1; presence of *Salmonella* = 1; high concentration of *Salmonella* = 1; and inflammatory cells infiltration = 1 (Argüello et al., 2018).

**Table 1**
Ingredients and chemical composition of the diet fed to pigs.

| Ingredient | Composition (%) |
|---|---|
| Corn | 73.74 |
| Soybean meal 44 % | 22.20 |
| Soybean oil | 0.86 |
| L-Lysine-HCl | 0.17 |
| Dicalcium phosphate | 1.15 |
| Limestone | 0.88 |
| Vitamin-mineral premix* | 0.50 |
| NaCl | 0.50 |
| Calculated composition | |
| Metabolizable energy (kcal/kg) | 3,300 |
| Crude protein | 16.0 |
| Lysine | 0.95 |
| Methionine | 0.26 |
| Calcium | 0.66 |
| Total protein | 0.56 |

* Values supplied per kilogram of premixed feed concentrations: vitamin A, 5000,000 IU; vitamin E, 1000 mg; vitamin $B_1$, 150 mg; vitamin $B_2$, 300 mg; vitamin $B_{12}$, 1500 mg; niacin amide, 1500 mg; DL-calcium pantothenate, 1000 mg; folic acid, 200 mg; vitamin H, 10 mg; choline chloride, 2000 mg; manganese, 3800 mg; zinc, 1500 mg; iron, 4000 mg; Cu, 500 mg; I, 250 mg; Co, 100 mg; Mg, 200 mg.

### *Quantification of cytokines in gastrointestinal tissue*

Reverse transcription (RT)-PCR was performed to quantify the cytokine expression levels in the collected samples of the two experimental groups. The expression levels of the three IL genes (IL-10, IL-17A, and IL-18) related to the immune response against *Salmonella* infection were compared between the two groups. RNA isolation, complementary DNA synthesis, and quantitative RT-PCR were performed according to a previous study (Yi et al., 2023) (Table 2).

### *Microbial community analysis and measurement of short-chain fatty acid (SCFA) concentrations*

DNA was extracted from fecal samples of all experimental pigs using a commercial kit (DNeasy PowerSoil Kit; Qiagen, Hilden, Germany). The V3-V4 hypervariable region of the bacterial 16S rRNA gene sequence was amplified and sequenced following the Illumina 16S metagenomic sequencing library preparation protocol. Paired-end sequencing was performed on an Illumina MiSeq Platform (Illumina, San Diego, CA, USA), and the resulting fastq reads were imported into a QIIME2 platform (version 2.0). Cutadapt was used to trim adapters (Phred score < 20). Subsequently, the trimmed reads underwent processes such as quality filtering, denoising, and merging, with the removal of chimeras through the use of the DADA 2. The sequencing data were arranged according to the two experimental groups. Finally, the results were analyzed using bioinformatics, as described in our previous study (Yi et al., 2022).

Concentrations of SCFAs, including acetate, propionate, and butyrate, were measured using an Agilent 6890 B gas chromatograph (Agilent Technologies, Santa Clara, CA, USA). Fecal samples were mixed with methanol at a 1:10 ratio and homogenized at 250 rpm for 3 h. After spinning down the homogenate, the supernatant was collected and

**Table 2**
Primer information and condition for quantitative real-time polymerase chain reaction.

| Primer | Target | Sequence (5′ → 3′) | Size (bp) | Annealing Temp ( °C) | Reference |
|---|---|---|---|---|---|
| **IL-10-F** | Interleukin 10 | GCCTTCGGCCCAGTGAA | 101 bp | 62 °C | Walsh et al. (2012) |
| **IL-10-R** | | AGAGACCCGGTCAGCAACAA | | | |
| **IL-17A-F** | Interleukin 17A | CCCTGTCACTGCTGCTTCTG | 57 bp | 62 °C | Ryan et al. (2012) |
| **IL-17A-R** | | TCATGATTCCCGCCTTCAC | | | |
| **IL-18-F** | Interleukin 18 | ACGACCAAGTCCTTTTCATTAACC | 85 bp | 63.6 °C | Bouwhuis et al. (2017) |
| **IL-18-R** | | TGAGGTGCATTATCTGAACAGTCA | | | |
| **ACTB-F** | Beta actin | CAAATGCTTCTAGGCGGACTGT | 75 bp | – | Walsh et al. (2012) |
| **ACTB-R** | | TCTCATTTTCTGCGCAAGTTAGG | | | |

IL, interleukin; ACTB, actin beta.

filtered using a 0.45 μm Teflon syringe filter. The 5-μL sample was injected into a 50 m × 0.32 mm × 0.5 μm HP-FFAP capillary column (Agilent Technologies, USA). The injector was operated at 220 °C, and the detector was operated at 250 °C with a split ratio of 10:1. Nitrogen was used as a carrier gas at a flow rate of 1.0 mL/min. The column temperature was programmed as follows: initial temperature was maintained at 100 °C for 10 min, followed by an increase of 10 °C/min to 250 °C for 3 min.

### *Statistical analysis*

The unpaired Wilcoxon rank-sum and paired Wilcoxon signed-rank tests were used to compare the alpha diversity indices of the fecal microbiota composition between the ST and NC groups at 14 dpi and between the two time points (0 and 14 dpi), respectively. Beta diversity was analyzed by principal coordinate analysis (PCoA) of matrix distances using the Bray–Curtis dissimilarity index with QIIME software, version 2.0 (Knight and Caporaso Labs, AZ, USA). Linear discriminant analysis (LDA) effect size (LEfSe) analysis was also conducted using QIIME software, version 2.0. The taxa with a significant difference ($p < 0.05$) between both groups and those with an LDA score of $\geq 2.0$ were considered statistically significant and used to build an LEfSe. The specific changes in average daily gain (ADG), histopathological lesion scores, and SCFA concentrations between the two experimental groups were compared with the Student's *t*-test using SPSS software version 26.0 (IBM Corp., Armonk, NY, USA).

## Results

### *Growth performance, S. Typhimurium detection, and histopathological lesions*

The ADG and *S.* Typhimurium detection rates were compared between the two experimental groups. The ADG during the experimental period was lower in the ST group (0.07 kg/d) than in the NC group (0.18 kg/d) (Fig. 1A). The pigs in the ST group started to shed *S.* Typhimurium from 2 (100 %, 6/6) to 11 dpi (50.0 %, 3/6) (Fig. 1B). At 14 dpi, *S.* Typhimurium was most prevalent in the colon (100 %, 6/6), followed by the cecum (83.3 %, 5/6), jejunum (50.0 %, 3/6), and ileum (16.7 %, 1/6) (Fig. 1C). The total average histopathological lesion score was significantly higher in the ST group ($3.6 \pm 1.3$, $p < 0.001$) than in the NC group ($1.3 \pm 1.0$) ($p < 0.001$) (Fig. 2). In the ST group, the average lesion score was highest in the cecum ($4.5 \pm 0.5$, $p < 0.001$), followed by the colon ($4.3 \pm 0.7$, $p = 0.032$), ileum ($3.5 \pm 0.8$, $p = 0.002$), and jejunum ($1.67 \pm 0.75$, $p = 0.539$).

### *Inflammatory cytokine expression*

The anti-inflammatory cytokine IL-10 gene expression level was significantly higher in the jejunum of the ST group ($0.76 \pm 0.14$-fold) than in that of the NC group ($0.02 \pm 0.02$-fold) ($p = 0.006$) (Fig. 3). Although no significant difference in pro-inflammatory cytokine IL-17A gene expression in the jejunum ($p = 0.104$), cecum ($p = 0.109$), and colon ($p = 0.160$) was observed between the two experimental groups, IL-17A gene expression in the jejunum tended to be higher in the ST group ($4.70 \pm 2.48$-fold) than in the NC group ($2.13 \pm 0.82$-fold). The relative RNA expression of the pro-inflammatory cytokine IL-18 gene was significantly higher in the jejunum tissue from the ST group ($0.41 \pm 0.11$-fold) than in that from the NC group ($0.02 \pm 0.02$) ($p = 0.017$). The IL-18 gene expression was also significantly higher in the colon tissue from the ST group ($0.73 \pm 0.05$-fold) than in that from the NC group ($0.02 \pm 0.01$-fold) ($p < 0.001$).

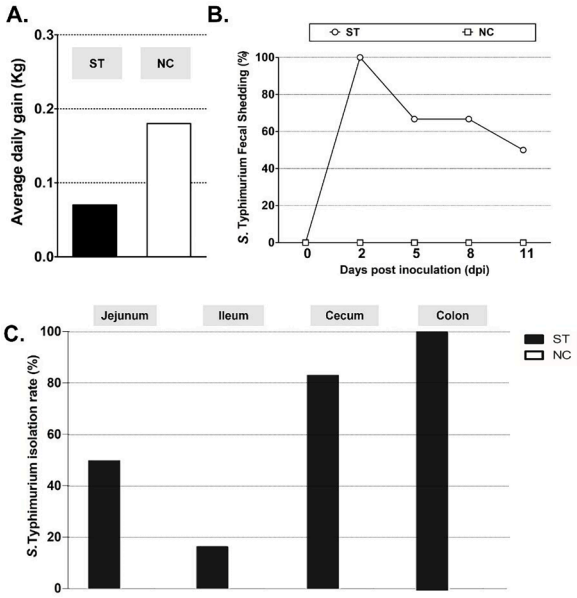


**Fig. 1.** Effect of *Salmonella* Typhimurium infection in 63-day-old weaned pigs. (A) Average daily gains of pigs in the *S.* Typhimurium-inoculated (ST) and negative control (NC) groups during the 14-day experimental period. (B) *S.* Typhimurium isolation rate (%) from fecal samples from the ST and NC groups until 11 days after *S.* Typhimurium inoculation (dpi). (C) *S.* Typhimurium isolation rate (%) from each organ culture in the ST group at 14 dpi. No growth is observed in the NC group.

### *Gut microbial diversity, composition, and fecal SCFA concentration*

To assess the effect of *S.* Typhimurium infection on the pig gut microbiota, an alpha diversity analysis was conducted between the ST and NC groups at 0 and 14 dpi (Fig. 4A). The Wilcoxon rank-sum test revealed that the average number of amplicon sequence variants (ASVs) and Chao1, Shannon, and Gini–Simpson indices tended to decrease in the ST group from 0 to 14 dpi but were rarely changed in the NC group (number of ASVs, $p = 0.48$ at 0 dpi and $p = 0.76$ at 14 dpi; Chao 1 index, $p = 0.48$ at 0 dpi and $p = 0.76$ at 14 dpi; Shannon index, $p = 0.76$ at 0 dpi and $p = 0.35$ at 14 dpi; and Gini–Simpson index, $p = 0.91$ at 0 dpi and $p = 0.067$ at 14 dpi.

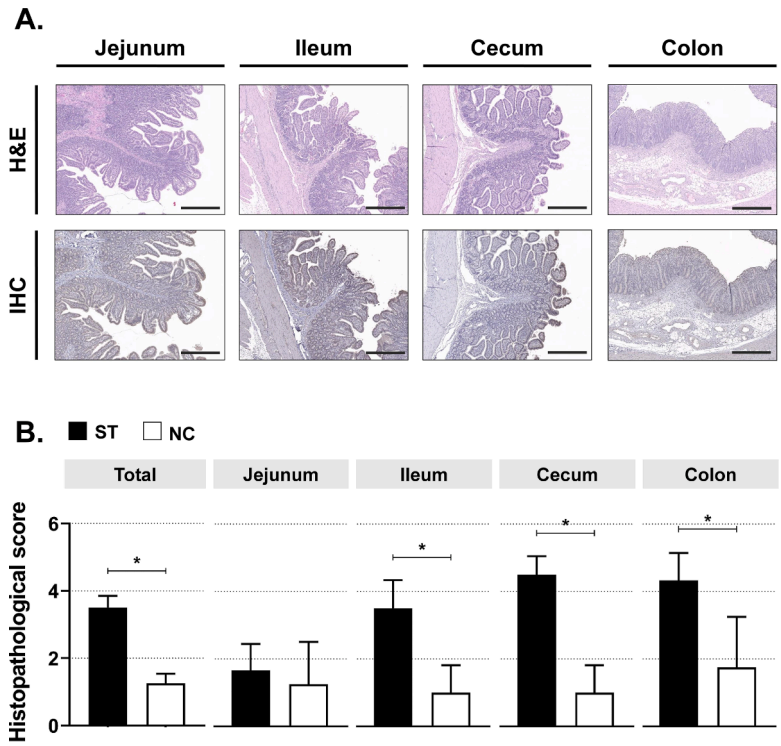


**Fig. 2.** Histopathological analysis results in this study. (A) Representative H&E and IHC (anti-*Salmonella* Typhimurium) staining in *Salmonella* Typhimurium-inoculated pigs. H&E; hematoxylin and eosin, IHC; immunohistochemical. (B) Histopathological scores of the intestinal organs. * $p < 0.05$.

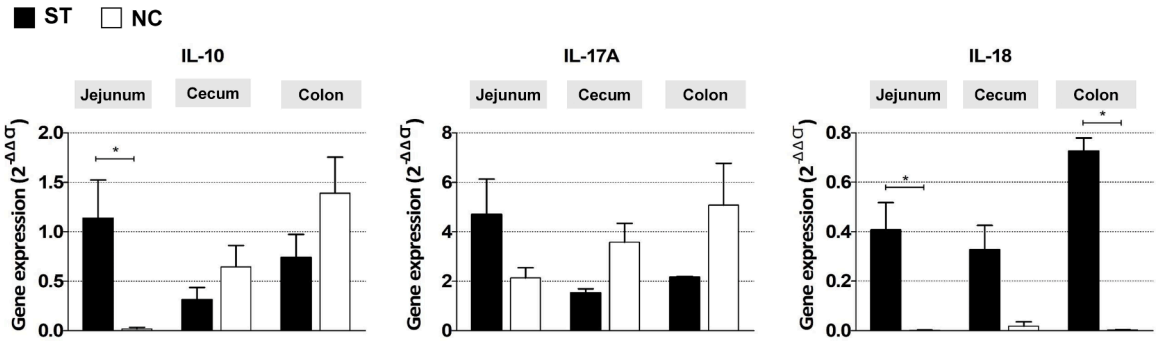


**Fig. 3.** mRNA expression of genes associated with inflammatory response (interleukin [IL]−10, IL-17A, and IL-18) in three intestinal tract tissues (jejunum, cecum, and colon) from the *Salmonella* Typhimurium-inoculated (ST) and negative control pigs (NC) groups at 14 days after *S.* Typhimurium inoculation. * $p < 0.05$.

To investigate the structure of the bacterial community, beta diversity was plotted using PCoA and a nonmetric multidimensional scaling plot based on the Bray–Curtis distance matrix. The gut bacterial community structure of the ST group shifted leftward, whereas that of the NC group shifted rightward along the PC1 axis on the PCoA plot at 14 dpi compared with that at 0 dpi (Fig. 4B). The LEfSe analysis clearly showed significant increases in the abundance of two genera, *Butyricicoccus* ($p = 0.0190$) and *Ethanoligenens* ($p = 0.0187$) in the ST group, but it showed significant decreases in the abundance of five genera, *Fusicatenibacter* ($p = 0.0247$), *Neglecta* ($p = 0.0136$), *Lachnospira* ($p = 0.0190$), *Paratractidigestivibacter* ($p = 0.0131$), and *Anaerobium* ($p = 0.0057$), in the ST group (Fig. 4C).

The concentrations of the three SCFAs, acetate, propionate, and butyrate, in fecal samples at 14 dpi were 1033.4 ± 120.4 μg/g, 789.3 ± 81.6 μg/g, and 697.3 ± 49.6 μg/g in the ST group, respectively, and 1168.1 ± 294.9 μg/g, 855.64 ± 175.4 μg/g, and 715.1 ± 78.2 μg/g in the NC group, respectively (Fig. S1).

## Discussion

The results of the current study showed that the ADG in the ST group was lower than that in the NC group, which is consistent with the findings of previous studies (Boyer et al., 2015; Liu et al., 2023). *S.* Typhimurium infection can result in an intestinal epithelium with a reduced villus height and crypt depth, resulting in nutrient malabsorption, which in turn decreases body weight gain in pigs (Fazelnia et al., 2021; O'Doherty et al., 2021; Rodrigues et al., 2021). A reduction in ADG is also frequently observed following pathogenic bacterial infections, including *S.* Typhimurium infection, which may be associated with activation of the acute phase response in weaned piglets (Huntley et al., 2018; Liu et al., 2023). Continuous activation of the immune system stimulated by *S.* Typhimurium requires additional energy supplementation, leading to reduced availability of amino acids and nutrients for growth, and also affecting the growth performance (body weight and ADG) of weaned piglets (Halas et al., 2011; Halas & Nochta, 2012). Decreases in herd productivity and economic losses in the pig industry highlight the importance of preventing *S.* Typhimurium

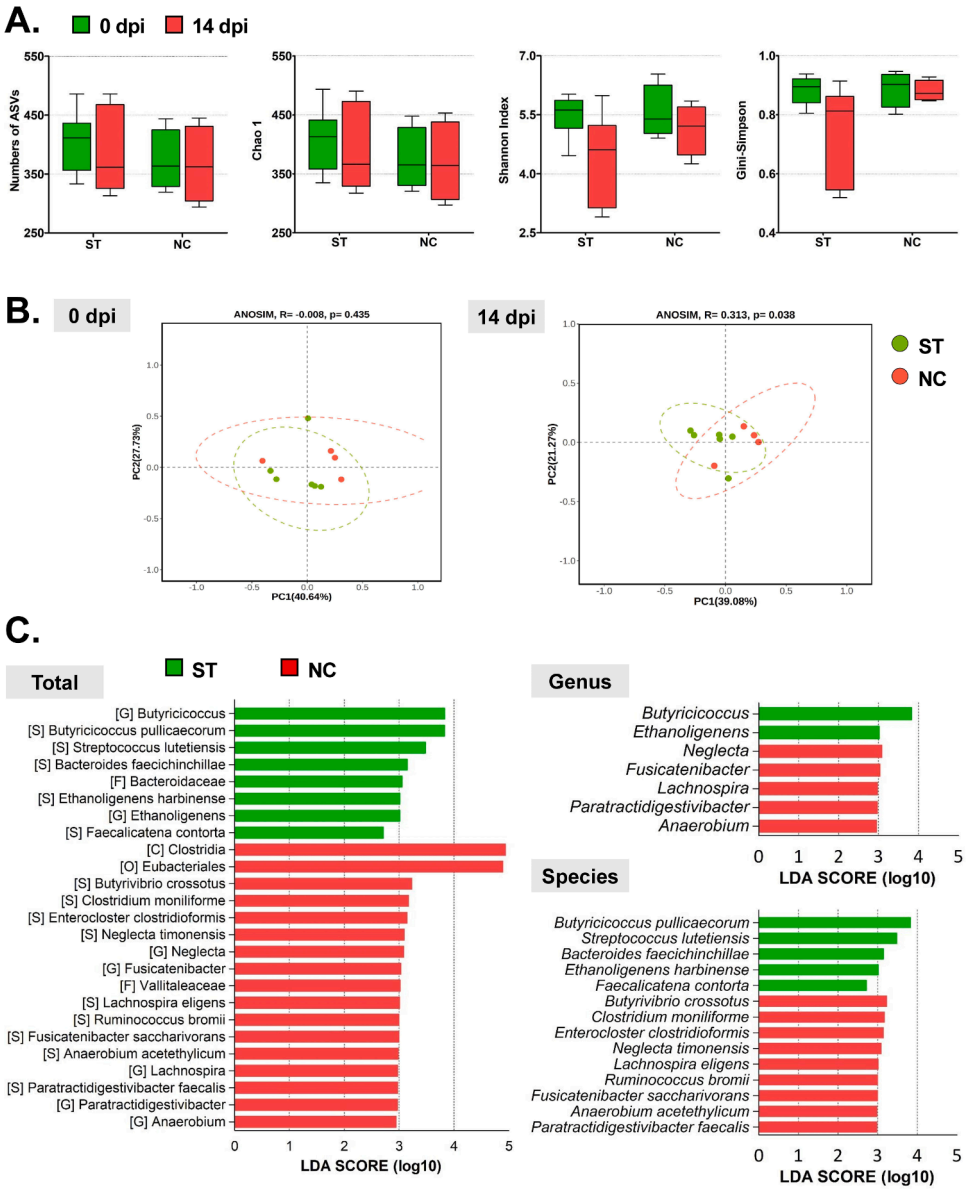


**Fig. 4.** Comparison of fecal microbiota diversity, composition, and short-chain fatty acid concentration in the *Salmonella* Typhimurium-inoculated (ST) and negative control (NC) pigs at 14 days after *S.* Typhimurium inoculation (dpi). (A) Comparison of fecal microbiota alpha diversity in the ST and NC groups between 0 and 14 dpi. (B) Gut microbiota community structure based on the Bray–Curtis dissimilarity matrix at 0 and 14 dpi. (C) Linear discriminant analysis effect size analysis reveals predicted biological effect sizes of differential taxa in the fecal microbiota between the ST and NC groups. The linear discriminant analysis (LDA) scores show a significant difference in the abundance and consistency of the detected bacterial taxa at the genus and species levels.

infections (Arce et al., 2014).

In this study, *S.* Typhimurium fecal shedding peaked at 2 dpi, followed by a gradual decrease, although it remained detectable until 11 dpi. The results highlighted that *S.* Typhimurium-infected pigs could shed the bacteria for approximately 9 days in the piggery, and spread it among the herd (Ivanek et al., 2012). With *Salmonella*-positive immunohistochemical reaction patterns, histopathological changes, such as epithelial villus shortening and inflammation, were commonly observed in the intestinal tissues of the ST group, suggesting that piglets could not fully recover by 14 dpi. These morphological and histopathological changes in the intestinal tissues of the ST group correlated with clinical signs, including mild to watery diarrhea (ST group: 5 of 6 pigs showed diarrhea; NC group: 1 of 4 pig showed diarrhea). Interestingly, we observed the lowest *S.* Typhimurium isolation rate in the ileum tissue samples. Although *S.* Typhimurium is known to preferentially colonize the ileum (Collado-Romero et al., 2012; Uribe et al., 2016), Collado-Romero et al. (2012) reported that gut epithelial damage is primarily observed in the ileal mucosa early after *S.* Typhimurium infection. The reduced *S.* Typhimurium isolation rate in the ileum tissue might be attributed to the timing of sample collection, which occurred later in the infection period (14 dpi), by which time a large number of *S.* Typhimurium bacteria had already been excreted.

Immunohistochemistry results showed dense colonization of *S.* Typhimurium throughout the four segments of the intestinal tract in the ST group, while none of the bacteria was found in the NC group, as expected. Hematoxylin-eosin staining showed villus shortening and inflammatory cell infiltration in each tissue of the ST group, even at 14 dpi. The binding of *S.* Typhimurium to intestinal cells triggers the production of inflammatory cytokines, such as IL-10, IL-17A, and IL-18 (Anderson & Kendall, 2017; Eckmann & Kagnoff, 2001), which is partly consistent with the substantial upregulation of IL-17A and IL-18 genes in the jejunum and that of IL-18 in the colon of the ST group at

14 dpi compared with the NC group. IL-18 is necessary to suppress *Salmonella* infection and stimulate T cells to produce IL-17, which contributes to *S.* Typhimurium clearance (Schmidt et al., 2020; Schulz et al., 2008). IL-10 suppresses the inflammatory response and prevents tissue damage (Splichalova et al., 2021), and it was also significantly increased in the jejunum of the ST group, where IL-17A and IL-18 genes were significantly expressed. However, these results had some limitations to establish a general conclusion. First, the present study focused only on three cytokines closely related to *S.* Typhimurium. Second, cytokine expression was investigated at 14 dpi, although *S.* Typhimurium fecal shedding peaked at 2 dpi, implying that most of the inflammation-related cytokines might have exhibited the most dynamic changes around 2 dpi.

Our results demonstrated that *S.* Typhimurium infection altered the microbiota composition and the relative abundance of certain taxa in the pig intestine, which is in accordance with previous findings (Argüello et al., 2018, 2019; Bearson et al., 2013). The LEfSe analysis showed that proportions of the genus *Butyricicoccus* and its subtaxon *B. pullicaecorum* increase immediately after weaning, which is associated with better feed efficiency in the pig gut according to previous studies (Holman et al., 2021; Quan et al., 2020; Tan et al., 2017). However, the significant increase of *Butyricicoccus* abundance did not affect the increase in ADG in this study; ADG was rather lower in the ST group than in the NC group. *Ethanoligenens* are cellulose-degrading and glucose-fermenting bacteria found in the pig gut (Huang et al., 2019; Li et al., 2020). The increased abundance of this bacterium is thought to be associated with the digestive capacity required to ferment solid feed and dietary fiber after weaning. We hypothesized that the following five genera would significantly decrease in the ST group; *Fusicatenibacter, Neglecta, Lachnospira, Paratractidigestivibacter*, and *Anaerobium*, which are specific and sensitive to *S.* Typhimurium infection. Previous studies have reported that *Fusicatenibacter* suppresses intestinal inflammation and is positively associated with total SCFA production in weaned piglets (Pang et al., 2021; Takeshita et al., 2016). Ma et al. (2022) reported that *Fusicatenibacter* continued to decrease significantly in human patients with early and advanced Crohn's disease, which is a gastrointestinal inflammatory disease. *Lachnospira*, especially *L. eligens*, has been reported to lead an increased production of acetate by increasing pectin fermentation (Chung et al., 2017; Pascale et al., 2022). The genus *Paratractidigestivibacter*, which consists of only one subtaxon, *P. faecalis* (previously *Olsenella faecalis*), produces lactic acid and has been identified in the gut of healthy human gut (Han et al., 2019). The genus *Anaerobium* contains only one species, *A. acetethylicum*, and degrades cellulose and produces butyrate by fermenting glycerol in the intestinal tract of pigs (Hidalgo & Puerta-Fernández, 2017; Patil et al., 2017). *Neglecta* and its subtaxon *N. timonensis* showed the highest positive correlation with ADG (Tran et al., 2018).

In conclusion, the overall study results suggest that *L. eligens* and *A. acetethylicum*, among the bacteria that are less abundant in the ST group, might act as important factors in the reduction of *S.* Typhimurium infection severity in pigs by producing SCFAs, such as acetate and butyrate. However, we could not isolate, culture, and characterize these bacteria because our study was based on metagenomics analysis. Future studies are needed to investigate the function and characteristics of these two bacteria in pigs with salmonellosis. The results of this study provide basic information about the microbiota alteration in *S.* Typhimurium-infected weaned piglets. These results could improve our understanding of swine salmonellosis, and help to develop alternative measures for preventing *S.* Typhimurium infection in future.

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## Ethics statement

All animal experiments were approved by the Institutional Animal Care and Use Committee of the National Institute of Animal Science, Republic of Korea (approval number: NIAS 2021-503, approval date: 30 March 2021).

## Data statement

The metagenomics sequencing data presented in this study have been deposited in the NCBI Sequencing Read Archive database under the accession number PRJNA1001520.

## CRediT authorship contribution statement

**Seung-Won Yi:** Data curation, Formal analysis, Investigation, Software, Visualization, Writing – original draft. **Han Gyu Lee:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft. **Eunju Kim:** Validation. **Young-Hun Jung:** Resources, Validation. **Eun-Yeong Bok:** Validation. **Ara Cho:** Validation. **Yoon Jung Do:** Validation. **Kyoung-Min So:** Validation. **Tai-Young Hur:** Resources, Validation. **Sang-Ik Oh:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.vas.2023.100324.

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