Time-course alterations of gut microbiota and short-chain fatty acids after short-term lincomycin exposure in young swine

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Abstract
Increasing evidence suggests that antibiotic administration causes gut injury, negatively affecting nutrient digestion, immune regulation, and colonization resistance against pathogens due to the disruption of gut microbiota. However, the time-course effects of therapeutic antibiotics on alterations of gut microbes and short-chain fatty acids (SCFAs) in young swine are still unknown. In this study, twenty piglets were assigned into two groups and fed commercial diets with or without lincomycin in the first week for a 28-day trial period. Results showed that 1-week lincomycin exposure (LE) did reduce the body weight on day 14 (p = 0.0450) and 28 (p = 0.0362). The alpha-diversity notably reduced after 1-week LE, and then gradually raised and reached the control group level in the second week on cessation of LE, indicated by the variation of Sobs, Chao, Shannon, and ACE index (p < 0.05). Beta-diversity analysis revealed that the distinct microbial cluster existed persistently for the whole trial period between two groups (p < 0.001). The relative abundance of most microbes including fiber-degrading (e.g., Agathobacter and Coprococcus), beneficial (e.g., Lactobacillus and Mitsuokella), or pathogenic bacteria (e.g., Terrisporobacter and Lachnoclostridium) decreased (LDA score > 3), and the concentration of SCFAs also diminished in the feces of 1-week lincomycin-administrated young swine, indicating that therapeutic LE killed most bacteria and reduced SCFA production with gut dysbiosis occurring. After the LE stopped, the state of gut dysbiosis gradually attenuated and formed new gut-microbe homeostasis distinct from microbial homeostasis of young pigs unexposed to lincomycin. The increased presence of potential pathogens, such as Terrisporobacter, Negativibacillus, and Escherichia-Shigella, and decreased beneficial bacteria, such as Lactobacillus and Agathobacter, were observed in new homeostasis reshaped by short-lincomycin administration (LDA score > 3 or p < 0.05), adversely affecting gut development and health of young pigs. Collectively, these results suggested that severe disruption of the commensal microbiota occurred after short-term LE or termination of LE in young swine.

Key points
- Therapeutic lincomycin exposure induced gut dysbiosis, killing most bacteria and reducing short-chain fatty acid production.
- Gut dysbiosis gradually attenuated and formed new homeostasis after lincomycin exposure stopped.
- The new homeostasis, increased Escherichia-Shigella etc. and decreased Lactobacillus etc., was potentially harmful to gut health.

Keywords Gut dysfunction · Antibiotic exposure · Fecal microbiota · Short-chain fatty acid · Young pigs

Introduction
Due to the bacteriostatic and bactericidal characteristics of antibiotics against pathogenic bacteria, antibiotics have made tremendous contributions to public health and low dose of antibiotics is also widely employed to promote growth and reduce costs of animal husbandry in animal production over the past decades. Recently, increasing evidence has demonstrated that antibiotics administration was documented to threaten animal health, injuring intestinal morphology and barrier, resulting in the dysfunction of intestinal immunity.
(Graversen et al. 2020; Looft et al. 2012; Zhang et al. 2020). Besides, the hazardous effect of antibiotic exposure on host health, such as *Clostridiodes difficile* infection-associated diarrhea (Post and Songer 2004), antibiotic-related diarrhea, and indigestion (Young and Schmidt 2004), was also obviously observed in previous studies. Moreover, indiscreet uses of antimicrobials lead to the production of superbugs with high antibiotic resistance (Gresse et al. 2017; Xiao et al. 2016). All these limitations of antibiotic use cause people to reconsider and restrict the application of antibiotics.

Despite increasing knowledge regarding the shortcomings of antibiotics use, the administration of antibiotics is commonly practiced for therapeutic treatment of mammals, especially young animals. Administration of antibiotics, which work via killing or suppressing pathogenic bacteria, induces imbalance of gut microbiota, causing the loss of colonization resistance (Stecher et al. 2013), and antibiotic-induced dysbiosis enables the expansion of pathogenic bacteria, which infect healthy hosts. The microbes, ecologically inhabiting the mammalian intestinal tract, can not only contribute to the maintenance of metabolic functions in the digestive process but also interact with the host to adjust the physiological and immunological states in digestive tract (Nieuwdorp et al. 2014; Qayed and Horan 2015). Also, the critical roles of commensal microbiota in maintaining epithelial integrity through providing colonization resistance against pathogens are reported (Stecher et al. 2013). Emerging evidence has displayed that early-life antibiotic exposure adversely affected the intestinal immunity, such as dysregulated vaccine immune response (Lynn et al. 2018), declined resistance to subsequent bacterial infection (Roubaud-Baudron et al. 2019), and altered maturation of innate intestinal immunity (Zhang et al. 2018), which was directly related to antibiotic-associated gut dysbiosis. Mammals have co-evolved with intestinal microbial communities that can shape development and adapt to environmental changes, including antibiotic perturbation or nutrient flux (Nobel et al. 2015). However, knowledge of the dynamic alterations of gut microbial communities in early-life, particularly in young animals after cessation of antibiotic administration, remains limited and requires further investigation.

Short-chain fatty acids (SCFAs), primarily produced via microbial carbohydrate fermentation in large gut, are considered beneficial metabolites interacting with intestinal microbiota to affect gut physiological functions, inflammation, and systemic metabolism (Koh et al. 2016). Previous studies exhibited that antibiotic administration attenuated the concentration of acetate, propionate, and butyrate in intestinal digesta or feces, responding to the alteration of gut microbiota (Gao et al. 2018; Zhang et al. 2020). However, a detailed understanding of dynamic changes of microbiota-derived SCFAs after cessation of antibiotic administration is still unclear. Different antibiotics have distinct susceptibility to different types of bacteria, which means that the impacts of different antibiotics on the composition of gut flora are also distinct. For example, colistin sulfate mainly acts on Gram-negative (G−) bacteria but has no effect on Gram-positive (G+) bacteria; the main function of enramycin is to kill G+ bacteria; some antibiotics are broad-spectrum antibacterial drugs, effective against both G+ and G− bacteria, such as oxytetracycline. Lincomycin, commonly used to treat a variety of G+ bacterial infections through inhibiting bacterial protein synthesis, is widely used in clinical practice for young children or in animal husbandry for diarrhea and fever of young animals due to its low price and no need for a skin test. Besides, lincomycin is often used as a substitute for penicillin, and its side effects are mainly gastrointestinal reactions. Since the limited information concerning the dynamic alteration role of gut microbiota with antibiotic exposure ceased is available, we objected to explore the time-course changes in fecal microbial composition and microbiota-derived SCFA after 1-week lincomycin exposure. Microbiome analysis and SCFA profiling revealed that 1-week lincomycin administration remarkably induced gut dysbiosis including reduced beneficial or pathogenic bacteria, and then microbiota reshaped and developed to the distinct cluster after lincomycin administration stopped.

**Materials and methods**

**Experimental pigs and study design**

Twenty weaned pigs (21-day old) housed at trial base of Kangjin town, Hulan district, Harbin, were randomly allocated into two groups (ten pigs in each group). The pigs in the test group (LM group) were administered with 1000 mg/kg lincomycin (purity ≥ 90%; Charoen Pokphand Bio, Pucheng, China) in feed for the first week, while the control group (Con group) pigs were without. After a 3-day adaptation period, the 28-day trial period officially started. All pigs were housed in separated pens with a plastic slatted floor, and the pens were cleaned on a daily basis to avoid disease occurrence. All animals were given ad libitum access to clean drinking water and basal feed (basal diet components detailed in Supplemental Table S1) during all periods. In order to ensure that the piglets could eat feed at any time, the feed was added twice a day at 8:30 AM and 2:30 PM. The amount of feed intake was recorded every day, and the body weight of each pig was recorded on day 0, 14, and 28 of the whole experiment. On the day 7, 14, 21, and 28 of the entire experiment, fresh fecal samples were collected directly from the rectum of two groups’ pigs through rectal massage, following loaded into 2-mL sterile tubes and immediately frozen in liquid nitrogen, and then stored at – 80 °C for...
sequencing of microbial 16S rDNA and analysis for SCFA quantification. The timeline is shown in Fig. 1A.

**DNA extraction, amplification, and sequencing**

Microbial community genomic DNA was extracted from feces samples using the E.Z.N.A.® soil DNA Kit (D5625-02, Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer’s instructions. The quality of extracted DNA was checked on 1% agarose gel (Supplemental Fig. S1A), and DNA quantity was determined with NanoDrop2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The extracted genomic DNA displayed suitable ratios of A260:A280 (1.8–2.0) and DNA concentration (20–300 ng/µL), meeting the requirements for subsequent sequencing.

The hypervariable region V3-V4 of the bacterial 16S rRNA gene was amplified by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA) with primer pairs 338F (5′-ACTCCTACGGAGGCAGCAG-3′) and 806R (5′-GGA CTAChVGGGTWTCTAAT-3′). The reaction parameters of PCR amplification was performed as follows: initial denaturation at 95 °C for 3 min, followed by 27 cycles of denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 45 s, and single extension at 72 °C for 10 min, and end at 4 °C. The PCR mixtures contain 5×FastPfu buffer 4 µL, 2.5 mM dNTPs 2 µL, forward primer (5 µM) 0.8 µL, reverse primer (5 µM) 0.8 µL, FastPfu Polymerase 0.4 µL, BSA 0.2 µL, template DNA 10 ng, and finally ddH2O up to 20 µL. PCR reactions were performed in triplicate. The PCR product was extracted from 2% agarose gel (Supplemental Fig. S1B) and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to manufacturer’s instructions and quantified using Quantus™ Fluorometer (Promega Corporation, Madison, WI, USA).

Purified amplicons were pooled in equimolar and paired-end sequenced on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database.

**Processing of sequencing data**

The raw 16S rRNA gene sequencing reads were demultiplexed, quality-filtered by fastp version 0.20.0 (Chen et al. 2018) and merged by FLASH version 1.2.7 (Magoc and Salzberg 2011) with the following criteria: (i) the 300 bp reads were truncated at any site receiving an average quality score of < 20 over a 50 bp sliding window, and the truncated reads shorter than 50 bp were discarded, and...
reads containing ambiguous characters were also discarded; (ii) only overlapping sequences longer than 10 bp were assembled according to their overlapped sequence, the maximum mismatch ratio of overlap region is 0.2, and reads that could not be assembled were discarded; (iii) samples were distinguished according to the barcode and primers, and the sequence direction was adjusted, exact barcode matching, 2 nucleotide mismatch in primer matching. Operational taxonomic units (OTUs) with 97% similarity cutoff (Edgar 2013; Stackebrandt and Goebel 1994) were clustered using UPARSE version 7.1 (Edgar 2013), and chimeric sequences were identified and removed. The taxonomy of each OTU representative sequence was analyzed by RDP Classifier version 2.2 (Wang et al. 2007) against the 16S rRNA database (Silva v138, https://www.arb-silva.de/) using a confidence threshold of 0.7.

Quantification of short-chain fatty acids

Gas chromatography (GC) was utilized to quantify feces SCFAs (including acetate, propionate, butyrate, valerate, isobutyrate, and isovalerate) according to the methods described by Tang et al. (2021). SCFA in the feces was extracted by ultrapure water, and 25% metaphosphoric acid was mixed with the extracts at a ratio of 1:9. After centrifugation, the mixture was passed through the 0.45-µm Milled-LG filter (Millipore, Billerica, MA, USA) and subjected for SCFA analysis with the Agilent 7890 N gas chromatograph (Agilent, Santa Clara, CA, USA).

Statistical analysis

Student’s t-test of the data on body weight, bacterial alpha-diversity indices (Sobs, Shannon, ACE, and Chao), and SCFAs were performed using the JMP software (JMP Version 10.0.0, SAS Institute, Cary, NC, USA) for Windows. p < 0.05 was regarded as statistically significant, while 0.05 < p < 0.1 was set as significant trend.

The principal coordinate (PCoA, beta-diversity) analysis based on the Bray–Curtis distance and ANOSIM test was employed using Majorbio I-Sanger Cloud Platform (www.i-sanger.com). The significant difference between the Con group and the LM group on different time points was tested by linear discriminant analysis effect size (LDA Effect Size, LEfSe), and the threshold of LDA score was 3.0. Statistical differences of key altered genera between two groups were calculated by the Mann–Whitney U test (nonparametric test) with p < 0.05 representing statistically significant and 0.05 < p < 0.1 representing significant trend.

Results

Growth performance

The initial body weight (5.47 ± 0.17 kg) of two groups’ pigs was comparable at the beginning of this study. After 7 days lincomycin exposure, the body weight was 5.42% or 9.23% lower than the Con group pigs on day 14 (p = 0.0450) or day 28 (p = 0.0362) (Fig. 1C). Besides, lincomycin exposure attenuated the average daily feed intake (Fig. 1B).

Evaluation of sequencing data

A total of 2,355,031 valid sequences from 64 feces samples were obtained with sequences ranging from 22,225 to 53,516 per sample after assembling and quality filtering. The normalized depth of 22,225 reads based on the minimum number of sample sequences was clustered into 1109 OTUs for all samples at a 97% sequence similarity value, further clustered into 239 genera, 96 families, 55 orders, 23 classes, and 15 phyla. The excellent coverage (> 0.999) and rarefaction curves (Supplemental Fig. S1C–E) indicated that most of the microbial diversity and bacterial communities in feces samples had been sufficiently captured.

Variation in alpha-diversity

Alpha-diversity is presented in Fig. 2 and Supplemental Fig. S2. The results showed that the microbial alpha-diversity (including Sobs, Chao, ACE, and Shannon indexes) in piglet feces did not change on the first three weeks of nursery period (p > 0.05), whereas the alpha-diversity (Sobs, p < 0.0001; Chao, p = 0.0002; ACE, p = 0.0003; Shannon, p < 0.0001) notably rose on the fourth week of nursery period (Fig. 2A–B and Supplemental Fig. S2A-B). One-week lincomycin exposure enormously decreased the microbial alpha-diversity (including Sobs, Chao, ACE, and Shannon indexes) in piglet feces (p < 0.0001), and the lower Sobs, Chao, ACE, and Shannon indexes were still observed after one week on cessation of lincomycin exposure (p < 0.0001) (Fig. 2A–B and Supplemental Fig. S2A–B). Yet no alterations of microbial alpha-diversity (including Sobs, Chao, ACE, and Shannon indexes) in feces between LM group pigs and Con group pigs were found after 2 weeks or 3 weeks on cessation of lincomycin exposure (p > 0.05, Fig. 2A–B and Supplemental Fig. S2A-B). After termination of lincomycin exposure, the Sobs, Chao, ACE, and Shannon indexes of fecal microbiota in LM group pigs gradually ascended with the time passing and reached a significant level (p < 0.05, Fig. 2A–B and Supplemental Fig. S2A–B).
Variation in beta-diversity

The PCoA results based on the Bray–Curtis distance and ANOSIM test at OTU level revealed that community structure of fecal microbiota was no clear difference on the first 3 weeks of pig nursery period, but distinct microbial community on the fourth week of nursery period was formed ($R = 0.4385$, $p < 0.001$, Fig. 3B and A). Compared with Con group pigs, 1-week lincomycin-exposed pigs exhibited a distinct cluster in feces microbiota ($R = 0.5123$, $p < 0.001$, Fig. 3D and A). Even on the first ($R = 0.6295$, $p < 0.001$, Fig. 3E and A), second ($R = 0.5020$, $p < 0.001$, Fig. 3F and A), and third weeks ($R = 0.6052$, $p < 0.001$, Fig. 3G and A) of cessation of lincomycin exposure, the fecal microbiota of the two groups still demonstrated two notably distinct cluster. After termination of lincomycin exposure, the community structure of fecal microbiota in LM group pigs gradually shifted with the time passing and reached a significant level ($R = 0.6779$, $p < 0.001$, Fig. 3C and A).

Comparison of microbial composition at phylum and genus levels

At phylum level, most abundant phyla were *Firmicutes* and *Bacteroidota*, which consists of more than 90% of total phyla with *Actinobacteriota* in fecal microbiota (Supplemental Fig. S3A). One-week lincomycin exposure increased the relative abundance of *Firmicutes* and decreased *Bacteroidota* in fecal microbiota, but none of them reached a significant level ($p > 0.05$, Supplemental Fig. S3A-C). The increment of *Firmicutes* ($p = 0.0181$) and decrement of *Bacteroidota* ($p = 0.0136$) were still observed on the third week after the termination of lincomycin exposure, which reached a significant level (Supplemental Fig. S3A-B and D).

At genus level, the top ten abundance genera in pig feces were *Prevotella*, *Clostridium_sensu_stricto_1*, *Subdoligranulum*, *Blautia*, *Ruminococcus_torques_group*, *Prevotellaceae_NK3B31_group*, *norank_f_Muribaculaceae*, *norank_f_norank_o_Clostridia_UCG-014*, *Olsenella* and *Terrisporobacter*, which consists of ~50%

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**Fig. 2** Alpha-diversity. The box plot of Sobs (A) and Chao (B) index (OTU level) in feces after lincomycin exposure. *** indicates $p < 0.001$ between Con and LM group ($n = 8$ pigs/group). Boxes (x, y or a, b, c, d) of Con or LM group with different letters are significantly different at $p < 0.05$ among different time points, respectively.
of total genera (Fig. 4A). The alterations of the top 50 genera in relative abundance attracted more attention. Some relative abundance of genera notably modified after 1-week lincomycin exposure, but those genera restored normal mass after 3 weeks on cessation of lincomycin exposure (e.g., *norank_f_Butyricicoccaceae*, *norank_f_Ruminococcaceae*, *Catenibacterium*, and *Catenisphaera*; Fig. 4A–B). Although another part of genera did not alter after lincomycin exposure, the relative abundance of those genera was varied remarkably after 3 weeks on the termination of lincomycin exposure (e.g., *Ruminococcus_torques_group*, *norank_f_Muribaculaceae*, *Olsenella*, *Prevotella*, *Lachnospiraceae_NK4A136_group*; Fig. 4A–B). The relative abundance of most genera obviously diminished due to the lincomycin exposure, among which most of those genera achieved augment level (e.g.,...
**Alteration of specific microbiota**

LEfSe was employed to identify the specific taxa at genus level in the fecal microbiome that was notably associated with lincomycin exposure and the threshold of LDA score was 3.0. A total of 26, 43, 28, or 31 microbiotas were identified in feces of pigs exposed to lincomycin on day 7, 14, 21, or 28, respectively (genus level, LDA score > 3, Supplementary file 1, Figs. 5A and 6A). The top five abundant microbiotas of identifies taxa in feces on day 7 were *Blaumia* (6.28%), *Prevotellaceae_NK3B31_group* (4.43%), *Lactobacillus* (4.30%), *Clostridium_sensu_stricto_1* (4.09%), and *Ruminococcus* (2.51%) (Fig. 5C). As shown in Fig. 5A–B,
most abundant microbiotas of identified taxa at genus level remarkably declined after 1-week lincomycin exposure, among which ten microbiotas (Agathobacter, Megasphaera, Phascolarctobacterium, Succinivibrio, Mitsuokella, Enorma, Terrisporobacter, Clostridium_sensu_stricto_1, Clostridium_sensu_stricto_6, and norank_f_Oscillosporaceae) with a tenfold decrement were observed. In contrast, a small number of bacteria with a significant increase in relative abundance varied from 3.4 to 8.4-fold, whereas the augmented level of Enterococcus was more than 40 times. More than 80% of altered microbiotas in feces on day 7 were derived from Firmicutes phylum, and most of them were from Clostridia class (Fig. 5B).

The most abundant microbiota of identified taxa were Prevotella (28.72%), Clostridium_sensu_stricto_1 (5.29%), and Lactobacillus (4.93%), which consists of ~50% of total microbiotas at genus level with Agathobacter (3.62%), unclassified_f_Prevotellaceae (2.02%), norank_f_Muribaculaceae (1.56%), Terrisporobacter (1.42%), unclassified_f_Oscillosporaceae (1.32%), and Alloprevotella (0.91%) on day 28 of whole trial (Fig. 6C). After 3 weeks on cessation of lincomycin exposure (day 28), only the relative abundance of two microbiotas (Eubacterium and Christensenellaceae_R-7_group) ascended by more than 10-fold, and other identified bacteria varied from 1.2-fold to 7.12-fold (Fig. 6A-B). After filtering out the bacteria...
with an abundance of less than 0.1% among the identified differential bacteria, we observed that 10 bacteria in the feces of lincomycin-exposed pigs were potentially beneficial bacteria (most of them were SCFA producers), including 5 downregulated genera (Agathobacter, Prevotella, Mitsuokella, Prevotellaceae_UCG-001, and Lactobacillus) and 5 upregulated genera (Eubacterium, norank_f_Muribaculaceae, Romboutsia, unclassified_f_Lachnospiraceae, and Christensenellaceae_R-7_group) (Fig. 6B). Besides, 6 pathogenic microbiotas (Oscillospira, Terrisporobacter, Negativibacillus, Clostridium_sensu_stricto_1, Alistipes, and Alloprevotella) were remarkably altered at genus level in the feces after 3 weeks on cessation of lincomycin exposure (day 28, Fig. 6B). Approximate 90% of altered microbiotas in feces on day 28 were derived from Firmicutes (71%) and Bacteroidota (19%) phylum, and most of them were from Clostridia (58%) and Bacteroidia (19%) class (Fig. 6B). The nonparametric test was utilized to further analyze the alterations of the well-recognized and relatively high abundant bacteria at each time point. One-week lincomycin exposure noticeably reduced the relative abundance of Terrisporobacter and Clostridium_sensu_stricto_1 (p < 0.001),
whereas the relative abundance remarkably ascended on day 14, 21, and 28 with lincomycin exposure ceased ($p < 0.05$) (Fig. 6D). Besides, 1-week lincomycin exposure augmented the relative abundance of *Escherichia-Shigella* ($p = 0.0339$) and *Negativibacillus* (not reach statistical significance level), and the upward trend of these two bacteria had decreased in the first week after the termination of lincomycin exposure; the relative abundance of *Escherichia-Shigella* and *Negativibacillus* descended in the second week after stopping lincomycin exposure, but the decline of those two bacteria notably reversed in the third week with lincomycin exposure ceased ($p < 0.01$) (Fig. 6D). The diminished *Lactobacillus* and *Agathobacter* were obviously observed after 1-week lincomycin exposure ($p < 0.05$), and the attenuated abundance of these two bacteria continued until the third week on cessation of lincomycin exposure (Fig. 6D).

**Predicted function of microbiota analysis**

To better comprehend the effect of lincomycin exposure on fecal microbiota in pigs, Tax4Fun analysis was used to predict the functional role of altered microbiota in feces. The results of predicted function demonstrated that 55, 4, 5, or 68 functional pathways at level 3 were obviously altered between LM and Con group pigs on day 7, 14, 21, or 28, respectively ($p < 0.05$, Supplementary file 2). The relative abundances of genes involved in metabolism-related pathways were noticeably altered after 1-week lincomycin exposure (day 7), such as amino acid metabolism (e.g., alanine, arginine, histidine, valine, and glutathione), carbohydrate metabolism, lipid metabolism, nucleotide metabolism (e.g., purine and pyrimidine), and protein digestion and absorption (Supplemental Fig. S4A, Supplementary file 2). Carbohydrate metabolism-related pathways or lipid metabolism-related pathways were regulated in the first or second week on cessation of lincomycin exposure (Supplementary file 2). In contrast, we observed that metabolism-related pathways, such as amino acid metabolism, carbohydrate metabolism, energy metabolism, and lipid metabolism, were remarkably regulated in third week with lincomycin exposure ceased (Supplemental Fig. S4B, Supplementary file 2).

**Short-chain fatty acid production**

Based on the changes of fecal microbiota, further investigation of SCFA quantification on different time points after 1-week lincomycin exposure was completed and depicted in Fig. 7. One-week lincomycin exposure did diminish the concentration of acetate ($p = 0.0391$), valerate ($p < 0.0001$), isovalerate ($p = 0.0079$), isobutyrate ($p = 0.0060$), and total SCFA ($p = 0.0162$), and decreased trend in butyrate level ($p = 0.0639$) was obviously found in the lincomycin exposed pigs. Despite the lower level of propionate from the trend point of view in lincomycin exposed pigs on day 7, no significant difference was found ($p = 0.2162$). The concentration of isovalerate and isobutyrate restored normal level on cessation of lincomycin exposure, so no alteration was detected among the two groups on day 14, 21, or 28 ($p > 0.05$). In contrast, the lower concentration of acetate (D14, $p = 0.0238$; D21, $p = 0.0644$), propionate (D14, $p = 0.0431$; D21, $p = 0.0186$), or total SCFA (D14, $p = 0.0273$; D21, $p = 0.0866$) from feces of lincomycin exposed pigs was still maintained in the first or second week after the end of lincomycin exposure, but the downward trend of these SCFAs disappeared in the third week with lincomycin exposure ceased (D28, $p > 0.05$). The concentration of butyrate ($p = 0.0893$) and valerate ($p = 0.0793$) had a declined trend in the first week with lincomycin exposed ceased, and the decrement was abolished in the next 2 weeks ($p > 0.05$).

**Discussion**

The present study targeted to seek the dynamic alterations of gut microbiota after therapeutic doses of lincomycin administration, and we found that 1-week lincomycin exposure induced the imbalance of fecal microbiota, leading to shaping distinct microbial clusters on cessation of lincomycin exposure. Our present results demonstrated that descent of body weight of young pigs in the first or third week on cessation of 1-week lincomycin exposure (1 g/kg feeding) was notably observed, in accordance with previous study that 1-week lincomycin administration (1 g/L water) reduced the body weight of young mice (Zhang et al. 2020). In addition to the decrement of feed intake in this study, the reason for weight loss might be that therapeutic dose (high dose) of lincomycin administration injured jejunal morphology and reduced the villi height of young animals (Zhang et al. 2020), negatively affecting the absorption and transportation of nutrient.

Gut dysbiosis, also called imbalance of gut microbiota, is mainly caused by loss of microbial diversity and alterations in microbial composition, bacterial activity, or microbial distribution (Stecher et al. 2013; Wilkins et al. 2019). Previous researches report that the loss of microbial diversity and the changes of microbial composition and relative abundance were induced by lincomycin treatment (Jo et al. 2021; Qi et al. 2019; Zhang et al. 2020). In accordance with previous studies, present results displayed that therapeutic lincomycin administration attenuated microbial diversity and the fecal microbiota obviously separated into distinct cluster in LM pigs compared with Con pigs, indicating that the microorganism environment was disrupted by lincomycin and gut dysbiosis might happen. A large and rapid population of the inhibited or
destroyed gut microbiota increased with lincomycin exposure ceased, and the alpha-diversity was restored to normal level after 2 weeks on cessation of lincomycin exposure. Nevertheless, the obviously continuing changes of microbial composition and microbial abundance had happened, which suggested that gut microbes were reshaped by short-term lincomycin administration in young animals. Altogether, the time-course variation of fecal microbes served as an evident characteristic shift pattern of microbes after short-term therapeutic dose of lincomycin exposure in young animals.

At genus level, the relative abundance of most microbiota was remarkably reduced after short-term therapeutic dose of lincomycin exposure in young animals, no matter beneficial (e.g., Lactobacillus, Agathobacter, and Coprococcus) or harmful bacteria (e.g., Terrisporobacter, Clostridium sensu stricto_1, and Clostridium sensu stricto_6). Among these notably declined microbes, genera Agathobacter (Bevilacqua et al. 2016; Horvath et al. 2021), Coprococcus (Li et al. 2019b), Megasphaera (Torres-Pitarch et al. 2020; Wei et al. 2020a), Phascolarctobacterium (Ferrulli et al. 2021; Han et al. 2020), Prevotellaceae_NK3B31_group (Shang et al. 2021; Wang et al. 2020), Succinivibrio (Cusco et al. 2021; Li et al. 2019a), and Mitsuokella (Onarman Umu et al. 2018; Zhang et al. 2019) are widely known symbionts for the degradation of dietary fiber, which is vital to gut health and immunity. On the one hand, colonization resistance of gut fiber-degrading bacteria constituted the first line of defense against invading pathogens, positively affecting gut health and immunity (Desai et al. 2016). On the other hand,
SCFAs, the usual product of fiber degradation, not only could supply energy to reduce the waste of nutrients but also severed as signaling metabolites derived from microbiota interacting with microbiota to affect gut health, inflammation and metabolic homeostasis (Yao et al. 2020; Zhou et al. 2020). Our study exhibited that the lower concentration of SCFAs (including acetate, butyrate, valerate, isobutyrate, and isovalerate) was observed in young pigs exposed to short-term lincomycin and similar results were also observed in mice (Zhang et al. 2020). Among which, the decrement of acetate was closely related to the decreased acetate-producing bacteria Prevotellaceae_NK3B31_group (Shang et al. 2021; Wang et al. 2020) and Succinivibrio (Cusco et al. 2021; Li et al. 2019a), whereas the descended butyrate was strongly associated with the descent of butyrate-producing bacteria Agathobacter (Bevilacqua et al. 2016; Horvath et al. 2021), Coprococcus (Li et al. 2019b), and Megasphaera (Torres-Pitarch et al. 2020; Wei et al. 2020a). Besides, the relative abundance of high abundant and beneficial microbiota Lactobacillus, also known as acetate-producing bacteria, was enormously diminished, which responded to the lower acetate concentration. These negative results indicated that the attenuation of fiber-degrading bacteria and Lactobacillus induced by short-term lincomycin exposure in young animals was harmful to gut health and the maintenance of immune function.

The results in accordance with expectation was that the restraint of well-recognized pathogenic bacteria Trevisporobacter (Chen et al. 2021), Lachnocolostridium (Hou et al. 2020; Wei et al. 2020b), Clostridium_sensu_stricto_1 (Fu et al. 2020; Shang et al. 2021), and Clostridium_sensu_stricto_6 (Liu et al. 2021) and unknown functional bacteria norank_f_Oscillospiraceae, unclassified_f_Prevoetellaceae, etc. was notably observed after short-term lincomycin treatment. Nonetheless, the relative abundance of potentially pathogenic bacteria Enterococcus was actually higher after lincomycin administration, which usually occurred in repeated antibiotic treatments, negatively affecting gut health of young animals (Comerlato et al. 2013; Ivanov et al. 2009). Further Tax4Fun analysis for functional prediction of microbiota revealed that the enriched pathways related to carbohydrate (including fiber degradation), amino acid, lipid, and nucleotide metabolism were obviously altered after lincomycin administration. Many amino acids considered signaling molecules are required to maintain gut health, regulating intestinal immunity, such as arginine synthesized from glutamine which could enhance intestinal epithelial barrier function (Costa et al. 2014). All these data indicated that the gut dysbiosis (including decreased fiber-degrading bacteria and Lactobacillus) and the attenuation of microbiota-derived SCFA might be harmful to gut health after short-term lincomycin administration.

After lincomycin administration ceased, we continued to detect the changes in fecal microbiota and microbiota-derived SCFA for 3 weeks. The interaction of different microbes is critical to the recovery of gut ecosystems with severe perturbations. Typically, some keystone species are needed to trigger a chain of food-net interactions, resulting in the recovery of gut microbiota, and a previous study claimed that key species related to specific carbohydrate-degradation and energy-production pathways supported gut ecosystems recovery (Chng et al. 2020). Our results demonstrated that the enormous fold-change of altered microbiota (including SCFA-producing bacteria) and lower SCFA concentration induced by lincomycin exposure gradually became small over time after lincomycin exposure stopped, but compared with Con pigs, LM pigs showed the distinct microbial community on cessation of lincomycin exposure, indicating that the process of initial gut ecosystems recovery to reshape new gut homeostasis happened with lincomycin exposure ceased. Following microbial changes after the end of lincomycin exposure could reflect the microbial adaptation to the new gut environment (Zhao et al. 2019). Confirming this possibility, a recent study depicted a model that showed for the recovery of the Palleja et al. (2018) microbial communities from antibiotics among which there was a transition to a “new alternative stable state” (Shaw et al. 2019).

Nevertheless, the new host-microbe homeostasis reshaped by short-term lincomycin administration does seem unfriendly to gut health. Genus Agathobacter can utilize acetate as a substrate to produce butyrate (Bevilacqua et al. 2016; Horvath et al. 2021) and genus Lactobacillus produces numerous advantageous organic acids that can be converted into butyrate (He et al. 2019), which have beneficial effects on the host. Our results displayed that the attenuation of these two beneficial bacteria was observed in the whole experimental time. Clostridium_sensu_stricto_1, generally considered pathogenic bacteria, is associated with inflammation (Shang et al. 2021) and pediatric diarrhea, as well as mucosal injuries in the colon (Fu et al. 2020), and Trevisporobacter is an emerging anaerobic pathogen and acetogenic bacterium (Chen et al. 2021), which are harmful to the gut. One-week lincomycin treatment notably diminished genera Clostridium_sensu_stricto_1 and Trevisporobacter level in feces of young pigs. However, the relative abundance of these two pathogenic bacteria immediately rebounded and continued to keep high abundance as soon as the lincomycin treatment disappeared. Besides, pathogenic bacteria Negativibacillus and Escherichia-Shigella associated with gut dysbiosis and the pathogenesis of inflammatory bowel disease (Bassett et al. 2015; Wang et al. 2021) were dramatically ascended in this research after lincomycin treatment at first, the cessation of lincomycin disturbed their relative abundance. After the dynamic variation, the relative
abundance of *Negativibacillus* and *Escherichia-Shigella* undergoes drastic ascent in the third week on cessation of lincomycin administration. Moreover, further Tax4Fun analysis suggested that predicted functional alteration of fecal microbial communities with time on cessation of lincomycin administration was related to lipid, carbohydrate, and amino acid metabolism pathways. Collectively, our data indicated that gut dysbiosis induced by lincomycin exposure still had relatively long-term adverse effects on gut health after stopping lincomycin exposure.

Intriguingly, the alterations of two body weight-related microbiotas were identified, which might partly explain the decrement of body weight after lincomycin administration. Pathogenic inflammation-related bacteria *Oscillospira* appreciably enhanced in the third week on cessation of lincomycin treatment, which was strongly associated with host metabolic disorders and body weight changed (Yang et al. 2018; Zha et al. 2021). Simultaneously, the effect of *Christensenenella*, the low abundant (less than 0.01%) and highly heritable (transmissible from parent to offspring) bacteria, on the mitigation of body weight gain has been proved (Sonnenburg and Backhed 2016), confirming that low abundant microbiota can impact host physiology (Goodrich et al. 2014), and genus *Christensenella* also notably augmented with low body weight in this trial on day 28.

In conclusion, the imbalance of gut microbes and microbiota-derived SCFA caused by short-term lincomycin exposure occurred in young pigs, demonstrating the drastic decrement in the relative abundance of numerous microbiotas including fiber-degrading bacteria (e.g., *Agathobacter* and *Coprococcus*), beneficial bacteria (e.g., *Lactobacillus* and *Mitsuokella*), or pathogenic bacteria (e.g., *Terrisporobacter* and *Lachnoclostridium*). After the lincomycin exposure stopped, the state of gut dysbiosis gradually attenuated and formed new gut-microbe homeostasis distinct from microbial homeostasis of young pigs unexposed to lincomycin. The increased presence of potential pathogens, such as *Terrisporobacter*, *Negativibacillus*, and *Escherichia-Shigella*, and decreased beneficial bacteria, such as *Lactobacillus* and *Agathobacter*, were observed in new homeostasis reshaped by short-lincomycin administration, adversely affecting gut development and health of young pigs. Our study depicts the change rule of gut microbiota in young animals under the condition of short-term lincomycin treatment, providing basic data for evaluating the harmful impact on the young animal gut microbe. The limitation of this research is that only alterations of fecal microbes in the first 3 weeks after therapeutic antibiotic exposure were observed, not continuously follow the young animal to adulthood to view the long-term effects.

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**Author contribution** S.T., R.Z., and H.Z. designed the research; S.T., R.Z., S.Z., and D.S. conducted the research; S.T. and L.C. analyzed the data; S.T. and R.Z. wrote the paper and had primary responsibility for the final content; L.L. and B.X. provided the animals and expertise. All authors read and approved the final manuscript.

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**Data availability** The datasets used during the current study are available from the corresponding author on reasonable request. The raw data on fecal microbiota generated from sequencing during the current study are available in the NCBI Sequence Read Archive (SRA) database (Accession Number: PRJNA750589).

**Declarations**

**Ethics approval and consent to participate** All procedures used in this study were granted by the Experimental Animal Welfare and Ethical Committee of Institute of Animal Science of Chinese Academy of Agricultural Sciences (IAS2020-107).

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