Dietary supplementation with *Bacillus* mixture promotes the gut health of weaned piglets

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Original article

Keywords: Bacillus, Gut microbiota and metabolites, Intestinal morphology, Probiotics, Weaned piglets

Posted Date: December 30th, 2019

DOI: https://doi.org/10.21203/rs.2.19700/v1

Abstract

This study was conducted to investigate the effects of dietary supplementation with a mixture of *Bacillus* on the intestinal health of weaned piglets. We randomly assigned 120 piglets to three groups; a control group (basal diet), a probiotics group (supplemented with 4×10^9 CFU/g *Bacillus licheniformis-B. subtilis* mixture; BLS mix), and an antibiotics group (supplemented with 0.04 kg/t virginiamycin, 0.2 kg/t colistin, and 3000 mg/kg zinc oxide). All groups had five replicates with eight piglets per replicate. On days 7, 21, and 42 of the trial, intestine and digesta samples were collected to determine the intestinal morphology, gut microbiota and metabolites, and the expression of genes related to gut health. The results showed that the BLS mix decreased the jejunum crypt depth, increased the ileum villus height, and increased the jejunum and ileum villus height to crypt depth ratio. The BLS mix also increased the expression levels of *E-cadherin* and *Occludin* in the colon and pro-inflammatory cytokines and *TLR4* in ileum and colon. The BLS mix also increased Simpson's diversity index in the gut microbiota and the relative abundances of o*_Bacteroidetes* and f*_Ruminococcaceae*, but it decreased the relative abundances of *Blautia*, and *Clostridium*. Collectively, these findings suggested that dietary BLS mix supplementation efficaciously promotes intestinal health through the modulation of gut microbiota in weaned piglets.

Introduction

In commercial swine production, early weaning contributes to intestinal development, digestive, or immune dysfunction in weaned piglets, which leads to reduced feed intake and growth inhibition (Campbell et al., 2013). Antibiotics are used to control the incidence of infectious diseases and improve growth in weaned piglets (Kim et al., 2012). However, many countries banned antibiotics in the livestock due to the increasing resistance of pathogens and antibiotic residues in food (Toutain et al., 2016; Allen et al., 2014). Therefore, effective antibiotic alternatives are urgently required to reduce the dependence of the animal industry on antibiotics. Previous studies have focused on the development of novel alternatives to antibiotics, including probiotics (Hu et al., 2018), prebiotics (Tran et al., 2018), and synbiotics (Zhang et al., 2018). Among these alternatives, probiotics have a higher potential to act pathogen exclusion (Azad et al., 2018).

Previous studies indicated that *Bacillus spp*. possess anti-oxidant, against pathogens, and immuno-modulatory abilities (Elisashvili et al., 2019). *Bacillus spp*. also produces various digestive enzymes that stimulate peristalsis of the intestine, thereby improving nutrient digestion (Giang et al., 2011). Presently, *Bacillus spp*. are widely used as commercialized probiotic products for humans and animals (Cutting, 2011). A previous study showed that a mix of *B. subtilis* and *B. licheniformis* can improve the growth and immunological status of farmed tilapia (Abarike et al., 2018). Moreover, dietary supplementation with B. *subtilis* DSM32315 played a beneficial role in maintaining the intestinal barrier function and microflora balance of weaned piglets, and it improved their growth performance (Tang et al., 2019). Lan and Kim (2019) suggested that dietary supplementation with a *B. licheniformis* and *B. subtilis* complex in growing-finishing pigs increased digestibility and the fecal Lactobacillus counts and decreased the fecal NH_3 and total mercaptan emissions.

However, the *Bacillus licheniformis* and *B. subtilis* mixture (BLS mix), as with other strains of *Bacillus*, have rarely been studied as probiotics for weaned piglets. Furthermore, little is known about the effects of the BLS mix on the intestinal morphology and microflora composition of weaned piglets. Our previous study indicated that dietary supplementation with *Bacillus subtilis* decreased the diarrhea rate and improved body weight gain in piglets (Wang et al., 2019a). Thus, we hypothesized that dietary BLS mix supplementation will improve the intestinal development and health of weaned piglets, through the modulation of the gut microbiota. Therefore, the present study investigated the changes in intestinal morphological, microbiota communities, and bacterial metabolites in weaned piglets fed different diets with the *Bacillus licheniformis* and *B. subtilis* mixture by Illumina HiSeq 2500 sequencing and biochemical analysis, to provide new evidence for the mechanism of action of these probiotics.

Material And Methods

Experimental design and dietary treatments

A total of 120 healthy crossbred piglets (Landrace × Large white, 7.00 ± 0.5 kg body weight) were weaned at 25 days of age and fed a corn and soybean meal-based diet. After 3 days of adaptation, the piglets were randomly assigned to one of three groups: a blank control group (basal diet), probiotics group (supplemented with 4×10^9 CFU/g *Bacillus licheniformis-B. subtilis* mixture; BLS mix), or an antibiotic group (supplemented with 0.04 kg/t virginiamycin, 0.2 kg/t colistin, and 3 000 mg/kg zinc oxide). The composition and nutrient levels of the basal diet met the nutritional requirements for nursey piglets established by the National Research Council (2012), which are shown in Table S1. The experiments lasted for 42 days.

The probiotic mixture, *B. licheniformis* (B26) and *B. subtilis* DSM32315 (B21), were produced by fermentation; the conditions were 200 r/min stirring speed and 350 L/h throughput. The fermentation products were filtered with an organic ceramic membrane filtration system and mixed with mineral adsorbent at a ratio of 1:1. The final product was a dry powder that was counted by the plate counting method to determine the viable number and then was mixed in the diet.

Sample collection and preparation

On days 7, 21, and 42 of the trial and 12 h after the last feeding, piglets from each replicate close to average weight were slaughtered by electric shock (120 V, 200 Hz). The intestinal contents from each colon (10 cm from the posterior to the ileocecal valve) were collected and stored at -20 °C for analyses of the short-chain fatty acids (SCFAs), indoles, skatoles, bioamines, and the composition of the microbiota. Samples of the jejunum, ileum, and colon tissue (approximately 2 cm) were collected, washed with cold physiological saline, immediately frozen in liquid nitrogen, and stored at -80 °C for further analyses. The jejunum and ileum from all piglets were fixed with 4 % paraformaldehyde-PBS overnight, and then dehydrated and embedded in paraffin blocks. A 5 μm section was cut from each sample for histological analysis.

Intestinal histological examination

The sections were deparaffinized, hydrated, and stained with hematoxylin and eosin (H.E.). From each intestinal sample, the villus length, villus width, and crypt depth were measured at 10 visual fields, and the villus height/crypt depth ratio was calculated.

16S sequencing and bioinformatics analysis

Microbial genomic DNA was extracted from all samples using a HiPure Stool DNA Kit (Magen, Guangzhou, China) following the manufacturer's instructions. A multiplexed amplicon library covering the V3-V4 region of the 16S rDNA gene was PCR-amplified with the optimized primer sets for the Illumina HiSeq 2500 sequencing instrument. Each paired-end read was then spliced using the FLASH (Magoc and Salzberg, 2011) software (version 1.2.1) to obtain the original spliced sequence (Raw contigs). Raw tags were mass filtered using the Trimmomatic software (version 0.33) to obtain high-quality clean data. All chimeric sequences were removed by Uchime (Edgar et al., 2011) (version 4.2). The chimera-free sequences were processed with a standard QIIME 1.91 pipeline (Bokulich et al., 2013) and clustered into operational taxonomic units (OTUs) at a 97 % similarity threshold using an "Open-Reference" approach.

Alpha rarefaction was analyzed by Chao1, Shannon, and Simpson indexes (Chao and Lee, 1992). To decipher the difference in microbiota structure between groups, LEfSe (linear discriminant analysis effect size) was performed, and the cladogram was graphed with default parameters (Segata et al., 2011). To probe the microbial metabolism and predict metagenome functional content from the marker gene, PICRUSt was utilized to explore differences in the KEGG pathway between groups (Langille et al., 2013). Spearman correlation coefficients were calculated for the correlation between gut health and the change in microbiota to establish suitable microbial compositions for better gut health.

Bacterial metabolites in colonic contents

The colonic contents were collected, homogenized, and centrifuged at 1 000 g for 15 min, as described previously (Kong et al., 2016). The intestinal SCFAs, including straight-chain fatty acids (acetate, propionate, butyrate, and pentanoate) and branched-chain fatty acids (BCFA; isobutyrate and isopentanoate) were detected by gas chromatography, as described previously (Zhou et al., 2014). The bioamines, including putrescine, tryptamine, tyramine, spermidine, and spermine were measured by high-performance liquid chromatography, as described previously (Xu et al., 2014). Indoles and skatoles were analyzed as in Kong et al (2016).

Analysis of gene expression related to gut health

Gene expression was quantitated by real-time polymerase chain reaction (RT-PCR), as described by Su et al. (2018). Briefly, total RNA was isolated from colonic tissues using TRIzol (Invitrogen, Carlsbad, CA, United States) and reverse transcribed with a Prime Script RT Reagent Kit with gDNA Eraser (Takara, Dalian, China). RT-PCR was conducted with primers of the target genes (Table S2), as well as the reference gene β-actin, and fluoresce was monitored by the SYBR Green detection kit (Thermo Fisher Scientific, Waltham, MA, United States) on a 7900 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The RT-PCR conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 5 s, and annealing at 60 °C for 30 s. Relative gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008).

Statistical analysis

Intestinal morphology index, colonic metabolite, and genes expression were analyzed with a one-way ANOVA in SPSS 17.0 software (SPSS, Inc., Chicago, IL, United States). The data are presented as means ± SE and $P < 0.05$ indicates statistical significance. The alpha diversity indices, relative species abundances, and overall composition of gut microbiota were analyzed using the Kruskal Wallis test. Spearman correlation coefficient was used to assess the relationships between health parameters and relative abundances of genera. LEfSe were used to identify different taxa microbes using default parameters.

Results

Effect of BLS Mix on the intestinal morphology of weaned piglets

The intestinal morphology data are summarized in Table 1. On day 7 of the trial, dietary supplementation with antibiotics or BLS mix decreased the crypt depth ($P < 0.05$) and increased the ratio of villus height to crypt depth in the jejunum compared with the control group ($P < 0.05$). On day 7 and day 42, the villus height and ratio of villus height to crypt depth in the ileum increased in both the antibiotics and BLS mix groups compared with the control group ($P < 0.05$). Interestingly, an increase in the crypt depth was observed in the BLS mix group relative to the antibiotics group on day 21 ($P < 0.05$).

Effect of BLS Mix on the microbiota diversity of weaned piglets

The 16S rDNA V3-V4 region amplicon sequencing generated 843 766 high-quality reads, with an average of 56 251 reads (range 45 822 to 67 947) per sample. Alpha diversity was measured to detect the diversity and structure of the colonic microbial communities in the different treatment groups (Figure 1). The Simpson index did not differ throughout the experimental period ($P > 0.05$). However, compared to the control group, the Chao1 index of the BLS mix group was lower on day 7 ($P<0.05$), whereas the Shannon index was higher on day 21 ($P<0.05$). This illustrates that dietary supplementation with the BLS mix increased the colonic microbial diversity of the piglets.

Effect of BLS Mix on the microbial communities of weaned piglets

Taxonomic classification of the microbial composition of the colonic contents revealed that *Firmicutes*, *Bacteroidetes*, and *Tenericutes* were the dominant bacterial phyla for the whole trial period (Figures 2A). At the genus level, *Lactobacillus*, *Ruminococcaceae*, *Clostridiales*, and *Clostridiaceae* were the dominant strains (Figures 2B). On day 7 and day 21, *Lactobacillus* (regardless of treatment) was the dominant strain, but on day 42, *Ruminococcaceae* was the dominant strain in the BLS mix and control groups.

The differences in the microbial communities at the phylum and genus levels are shown in Figure 3. On day 7, *Ruminococcaceae* occurred at higher amounts in the BLS mix group than in the control group ($P < 0.05$). *Blautia* occurred at lower levels in the antibiotics and BLS mix groups than in the control group ($P < 0.05$). On day 21 of the trial, Antibiotics led to a decrease in *Lactobacillus* and an increase in *Tenericutes* and *RF39* compared to their relative abundances in the control group ($P < 0.05$). The BLS mix led to an increase in *Bacteroidales* compared to their relative abundances in the control group ($P < 0.05$). On day 42, *Prevotella* occurred at a lower level in the BLS mix group than in the control group ($P < 0.05$). *Clostridium* occurred at a higher level in the antibiotics and BLS mix groups than in the control group ($P < 0.05$).

Effect of BLS Mix on the microbial function of weaned piglets

The LefSe analysis was performed to confirm the different effects of the BLS mix on the intestinal microbiota in piglets (Figure 4). Significant differences in the colonic microbiota were found between the control, antibiotic, and BLS mix groups for the entire experimental period. These results confirmed a significant enrichment of *Ruminococcaceae* in the BLS mix group on day 7. On day 21, *Clostridia* was most abundant in the antibiotics group, and *Elusimicrobia*, *Sphaerocheatales*, and *Enterobacteriales* were most abundant in the BLS mix group. On day 42, *Epsilonproteobacteria* and *Campylobacterales* were enriched in the control group, and *Chloroflexi, Streptomycetaceae*, and *Sva0853* were enriched in the antibiotics group.

The PICRUSt algorithm was performed to assess the functional differences by plotting the different pathways against the KEGG database (Figure 5). On day 7, the pathways enriched by the intestinal microbiota of the BLS mix include African trypanosomiasis and ether lipid metabolism. On day 21, the pathways enriched by the intestinal microbiota of the BLS mix include terpenoids and polyketides, cofactors and vitamins, amino acid metabolism, and carbohydrate metabolism. On day 42, the pathways enriched by the

intestinal microbiota of the BLS mix include carbohydrates (such as pyruvate, citrate cycle, propanoate, and butanoate), lipids, amino acid metabolism (such as lysine, tyrosine, cysteine, and methionine), cofactors and vitamins, and glycan biosynthesis and metabolism.

Effect of BLS Mix on the gut metabolites of weaned piglets

As shown in Table S3, the concentrations of SCFA and bioamines in the colonic contents of the three treatment groups did not differ on day 7 of the trial ($P > 0.05$). The SCFA concentration on day 21 also did not differ (Table 2). On day 42, the concentration of isobutyrate and isovalerate decreased in the BLS mix group relative to the control group ($P < 0.05$), while the acetate concentration increased ($P < 0.05$; Table 2). The bioamines concentration of the colonic contents is shown in Table 2. On day 21, the spermine concentration increased in the BLS mix group relative to the control and antibiotics groups ($P < 0.05$). The skatoles concentration increased in the antibiotics group relative to the control and BLS mix groups ($P < 0.05$). However, the skatoles concentration decreased in the antibiotics and BLS mix groups relative to the control group on day 42 ($P < 0.05$).

Effect of BLS Mix on the intestinal health-related genes of weaned piglets

On day 7 of the trial, dietary supplementation with the BLS mix increased the mRNA level of *IL-2, IL-6, IL-1β*, and *TLR-4* ($P < 0.05$) and decreased the level of TNF-α ($P < 0.05$) in the ileum compared to the antibiotics group. BLS mix supplementation also increased the mRNA level of IL-2 in the jejunum compared to the antibiotics group ($P < 0.05$). On day 21, dietary supplementation with the BLS mix increased the mRNA level of *TLR4* ($P < 0.05$) and decreased the mRNA level of TNF-α ($P < 0.05$) in the ileum compared to the antibiotics group. On day 42 of the trial, an increase in the mRNA level of *TLR-4* in the ileum was observed in the BLS mix group relative to the control group ($P < 0.05$).

The mRNA levels in the colon contents of the piglets from different dietary treatments are shown in Table 3. Compared to the control group, dietary supplementation with the BLS mix increased the mRNA level of *Occludin, IL-6, IL-1β*, and *TLR-4* on day 7, and the level of *E-cadherin* and *IL-1β* on day 42 ($P < 0.05$). Dietary supplementation with antibiotics increased *E-cadherin*, *IL-2, Occludin, IFN-α, TLR-4*, and *TNF-α* on day 21 of the trial.

Relationships between gut microbiota, metabolite, and intestinal health-related genes

A spearman's rank correlation analysis was performed to evaluate the potential link between alterations in gut microbiota and health parameters of the weaned piglets (Figure 6). The genus *Prevotella* was positively correlated with spermidine and spermine ($P < 0.01$) and negatively correlated with acetate and propionate ($P < 0.05$). The phylum *Bacteroidetes* and genus *CF231* were positively correlated with tryptamine, spermidine, and spermine ($P < 0.01$) but negatively correlated with acetate ($P < 0.01$). The genus *Anaerovibrio* was positively correlated with spermine but negatively correlated with acetate, propionate, and butyrate. The order *Bacteroidetes* was positively correlated with spermine ($P < 0.05$). The phylum *Treponema* was positively correlated with tyramine, tryptamine, putrescine, and spermidine but negatively correlated with skatole ($P < 0.05$). The family *Erysipelotrichaceae* was positively correlated with spermidine, *TLR-4,* and *IL-1β* ($P < 0.05$). The genus *Clostridium* was positively correlated with spermidine and negatively correlated with acetate ($P < 0.05$). The order *Clostridiales* was positively correlated with the level of *Occludin* ($P < 0.05$). The phylum *Firmicutes* was negatively correlated with spermidine ($P < 0.05$). The family *Ruminococcaceae* was positively correlated with the levels of *Occludin* and *TLR-4* ($P < 0.05$) but negatively correlated with tyramine, tryptamine, and putrescine ($P < 0.05$). The order *RF39* and phylum *Tenericutes* were negatively correlated with tyramine, tryptamine, and putrescine ($P < 0.05$). The genus *Lactobacillus* was positively correlated with acetate ($P < 0.05$) but negatively correlated with spermidine, *E-cadherin*, and *IL-1β* ($P < 0.05$). The genus *Blautia* was negatively correlated with *IL-1β* ($P < 0.05$).

Discussion

Bacillus spp. is widely used as a commercialized probiotic product for humans and animals (Cutting, 2011) and possess pathogen exclusion, anti-oxidant, and immuno-modulatory abilities (Elisashvili et al., 2019). To our knowledge, few studies have reported the effects of dietary BLS mix supplementation on the intestinal morphology, microflora composition, and metabolites of weaned piglets. The present study analyzed changes in the intestinal morphological, microbiota composition, and metabolites of weaned piglets fed different diets, and our data show that BLS mix supplementation efficaciously promotes intestinal development and health through the modulation of gut microbiota.

The intestinal mucosal barrier is an important modulator of intestinal homeostasis, and intestinal morphology reflects mucosal integrity and injury (Blikslager et al., 2007). The villus height and crypt depth represent the ability of the intestinal epithelial cells to digest and absorb nutrients (Yin et al., 2019). Our results showed that dietary BLS mix supplementation can decrease the jejunum crypt depth, increase the ileum villus height, and change the ratio of villus height to crypt depth in the jejunum and ileum. These findings suggest that the intestinal mucosal barrier is intact and has strong nutrient digestibility and absorption capacity (Li et al., 2019). Recent studies suggested that the gut physiological barrier is formed by the epithelial cells, tight junction proteins, and intestinal secretions (Suzuki, 2013; Yan and Ajuwon, 2017), in which the *E-cadherin*, *Occludin,* and *ZO-1* are indicators for tight junction assembly, stability, and barrier function (Zihni et al., 2016). The results presented here showed that the BLS mix upregulated the expression of *E-cadherin* and *Occludin* in the colon, suggesting that the BLS mix enhanced the repair of intestinal damage and preserved the integrity of the intestinal mucosa.

Alpha diversity reflects the species diversity in a single sample by incorporating indices for species richness (Chao and Ace) and diversity (Shannon and Simpson) (DeSantis et al., 2006). Our findings are consistent with those of Wang et al. (2019b), who found that probiotics increase the Simpson's diversity index of the microbial ecosystem in piglets. *Firmicutes* and *Bacteroidetes* are regarded as the main microbiota phyla in the pig gut, regardless of probiotic or antibiotic use (Wang et al., 2019b). The present study showed that dietary supplementation with the *BLS mix* increases the relative abundances of *Bacteroidale* and *Ruminococcaceae* and decreases the abundances of *Blautia,* and *Clostridium. Bacteroidales* can degrade proteins and carbohydrates (Thomas et al., 2011) and activate the host's immune system (Mazmanian et al., 2008). *Clostridium* is closely related to protein fermentation and can increase the risk of diarrhea (Rist et al., 2014). The lower abundance of *Clostridium* in the probiotics group may explain our previous finding that dietary supplementation with *Bacillus Subtilis* decreased the diarrhea rate in the piglets (Wang et al., 2019a). *Ruminococcaceae* ferments cellulose and hemicellulose and produces SCFAs used for energy (Biddle et al., 2013). These findings suggest that the *BLS mix* may regulate gut community composition and improve gut health.

PICRUSt (Langille et al., 2013) can be used to investigate the functional differences in microbiota to determine the metabolic alterations caused by antibiotics or probiotics (Wang et al., 2019b). In this study, dietary BLS mix supplementation increased the metabolic pathways of cofactors/vitamin metabolism and carbohydrate metabolism on day 21 of trial, as well as the metabolic pathways of carbohydrate, lipid, amino acid metabolism, cofactors and vitamins, and glycan biosynthesis and metabolism on day 42. Vitamins and cofactors are critical for the bioconversion of nutrients to energy and for maintaining homeostasis (Hu et al., 2016; Sharma et al., 2019). The glycan biosynthesis and metabolism pathway is important for carbohydrate metabolism (Hu et al., 2016; Varki, 2017). Gao et al (2017) showed that feed-additive probiotics accelerate intestinal microbiota maturation. Therefore, our results suggest that dietary BLS mix supplementation might accelerate intestinal microbiota maturation by enrichment of the important metabolic pathways.

The SCFAs produced by the colonic microbes via the fermentation of indigestible fiber are important for gut integrity, glucose homeostasis, and immune function (Morrison and Preston, 2016). In our study, acetate and propionate were the major SCFAs produced in the colon, which was consistent with previous findings in pregnant Huanjiang mini-pigs (Kong et al., 2016). Acetate can inhibit pathogenic bacteria, and butyrate acts as a major energy source for colonic epithelial cells (Morrison and Preston, 2016). BCFAs are produced by microbes through the deamination and decarboxylation of amino acids (Le Roy et al., 2013). In this study, dietary BLS mix supplementation increased the acetate concentration and decreased the concentrations of isobutyrate and isovalerate. Rist et al (2013) suggested that BCFAs might function as indicators of the extent of protein fermentation and exert detrimental effects on the host. These findings suggest that the BLS mix may inhibit pathogenic bacteria, decrease protein fermentation, and improve gut health.

The colonic microbiota catabolizes nitrogenous compounds to putrefactive catabolites, such as bioamines, indoles, and skatoles (Kong et al., 2016). Bioamines are markers of potentially harmful metabolic pathways. However, spermine is essential for somatic cell growth (Davila et al., 2013). Our results showed that dietary BLS mix supplementation can increase the spermine concentration and decrease the skatole concentration. High levels of bioamines and skatole might be toxic to gut health (Yin et al., 2018). Thus, decreasing the concentration of these compounds via a dietary BLS mix supplementation may exert beneficial effects on gut health.

Cytokines play crucial roles in the regulation of the immune and inflammatory responses and the barrier integrity of the gut (Andrews et al., 2018). LPS-mediated induction of the TLR4 signaling pathway results in the activation of nuclear factor κB (NF-κB) and, therefore, the expression of pro- and anti-inflammatory cytokines. The piglets challenged with LPS upregulated their gene expression of pro-inflammatory cytokines in the jejunal mucosa, including *TNF-α, IL-1β, IL-6, IFN-γ,* and *IL-8*. Our findings showed that dietary BLS mix supplementation increases the level of pro-inflammatory cytokine and *TLR4* in the ileum and colon, suggesting that the BLS mix might activate the inflammatory pathway and improve autoimmunity. Shi et al (2019) showed that moderate activation of the TLR4 signaling

pathway promotes inflammation and repair of the intestinal epithelium in DSS-induced colitis. Thus, we suggest that the BLS mix might alter the composition of the gut microbiota and accelerate intestinal microbiota maturation by increasing the metabolic pathways, enhancing the repair of intestinal damage, preserving the integrity of the intestinal mucosa, and providing better mucosal immunity.

In conclusion, dietary supplementation with the BLS mix had beneficial effects in weaned piglets, improving intestinal integrity and providing better mucosal immunity. These effects may be regulated by the activation of metabolic pathways and by the alteration of the gut microbiota community composition.

Declarations

Ethics approval and consent to participate

The experimental design and procedures used in this study were reviewed and approved by the Animal Care and Use Committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences. The animal experiments and sample collection strictly followed the relevant guidelines.

Consent for publication

Not applicable.

Availability of data and material

All sequences analyzed in this study can be accessed in the SRA database under the accession number PRJNA589726 (https://dataview.ncbi.nlm.nih.gov/object/PRJNA589726?reviewer=jscimf1vmv52kf9 c5236l4jve).

Competing interests

Author Wenming Zhang was employed by the company Evonik Degussa (China). The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

This study was jointly supported by the National Key Research and Development Project (2017YFD0500503), National Natural Science Foundation of China (31772613 and 31572421), STS regional key project of the Chinese Academy of Sciences (KFJ-STS-QYZD-052), and the Key Research Program of the Chinese Academy of Sciences (KFZD-SW-219-2-3).

Authors' contributions

XD, TZ, WM, and YZ performed the experiments. XD and TZ performed the statistical analyses and wrote the manuscript. ZB and XF contributed to experimental concepts and design, provided scientific direction, and finalized the manuscript. All authors read and approved the final manuscript.

Acknowledgments

We thank the staff and postgraduate students of Hunan Provincial Key Laboratory of Animal Nutritional Physiology and Metabolic Process for collecting samples, and technicians from Key Laboratory of Agro-ecological Processes in Subtropical Region for providing technical assistance. We would like to thank Editage (www.editage.cn) for English language editing.

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Tables

Table 1 Effect of dietary BLS mix supplementation on intestinal morphology in weaned piglets (n=5)

Item	Jejunum			Ileum		
	Control	Antibiotics	Probiotics	Control	Antibiotics	Probiotics
Day 7 of trial						
VH(μm)	334.42±6.77	370.64±9.29	421.88±12.83	304.69±19.15^{b}	386.02±40.10^{a}	380.09±21.48^{a}
CD (μm)	291.98±31.40^{a}	218.00±10.30^{b}	246.73±7.63^{ab}	171.76±4.10	198.66±19.75	181.13±12.14
VH/CD	1.20±0.13^{b}	1.71±0.05^{a}	1.71±0.01^{a}	1.78±0.14^{b}	1.94±0.06^{a}	2.12±0.15^{a}
Day 21 of trial						
VH(μm)	357.82±15.15	440.79±51.64	452.42±16.00	362.35±7.42	389.18±32.53	414.61±26.22
CD (μm)	260.30±19.98	269.95±38.28	315.32±16.74	174.85±3.86^{b}	188.91±8.06^{b}	226.93±11.06^{a}
VH/CD	1.41±0.13	1.67±0.10	1.45±0.09	2.08±0.06	2.09±0.25	1.84±0.12
Day 42 of trial						
VH(μm)	408.59±4.41	476.16±19.93	439.19±11.75	338.67±7.07^{b}	385.19±21.46^{a}	453.39±60.52^{a}
CD (μm)	296.57±16.46	332.11±22.76	263.13±10.27	206.36±11.20	175.23±16.39	198.35±23.65
VH/CD	1.39±0.06	1.45±0.07	1.68±0.08	1.67±0.13^{b}	2.23±0.13^{a}	2.27±0.05^{a}

VH, villus height; CD, crypt depth; VH/CD, villus height/crypt depth.

Table 2 Effect of dietary BLS mix supplementation on gut metabolites in weaned piglets (n=5)

Items	Day 21 of trial			Day 42 of trial		
	Control	Antibiotics	Probiotics	Control	Antibiotics	Probiotics
Short-chain fatty acids (mg/g)						
Acetate	3.06±0.05	3.15±0.17	2.94±0.15	2.23±0.20^{b}	2.30±0.22^{b}	3.06±0.08^{a}
Butyrate	1.13±0.09	1.08±0.04	0.97±0.09	0.87±0.12	0.64±0.13	0.85±0.13
Isobutyrate	0.10±0.01	0.10±0.01	0.11±0.01	0.13±0.03^{a}	0.09±0.02^{ab}	0.07±0.00^{b}
Isovalerate	0.17±0.01	0.18±0.01	0.20±0.03	0.24±0.07^{a}	0.17±0.05^{ab}	0.11±0.01^{b}
Propionate	1.61±0.05	1.79±0.12	1.46±0.10	1.09±0.16	0.97±0.13	1.25±0.06
Valerat	0.21±0.03	0.19±0.02	0.17±0.03	0.22±0.07	0.15±0.04	0.12±0.01
Bioamines (μg/g)						
Phenylethylamine	0.07±0.05	0.04±0.03	0.34±0.14	0.04±0.04	0.17±0.06	0.08±0.06
Putrescine	2.15±0.73	4.70±3.05	2.13±1.21	15.44±1.60	13.98±3.80	14.88±1.43
Spermidine	3.78±1.06	4.53±0.93	2.65±0.43	21.23±1.38	20.83±3.96	17.86±1.88
Spermine	2.44±1.48^{b}	1.40±0.49^{b}	6.22±0.47^{a}	4.01±0.50	5.01±1.17	4.33±0.36
Tryptamine	0.13±0.03	0.20±0.18	0.05±0.03	1.38±0.34	1.29±0.36	1.30±0.29
Tyramine	0.37±0.09	0.87±0.52	0.31±0.09	3.09±0.54	3.07±1.84	3.12±1.24
Indole	2.66±0.48	2.21±0.32	4.37±0.87	3.70±0.90	3.78±0.95	3.54±1.21
Skatole	6.82±0.75^{b}	11.58±1.68^{a}	4.61±0.85^{b}	11.59±3.50^{a}	5.26±1.00^{b}	2.73±0.74^{b}

Table 3 Effect of dietary BLS mix supplementation on intestinal health-related genes in weaned piglets (n=5)

Items	Day 7 of trial			Day 21 of trial			Day 42 of trial		
	Control	Antibiotics	Probiotics	Control	Antibiotics	Probiotics	Control	Antibiotics	Probiotics
E-cadherin	1.00±0.08	1.19±0.23	1.22±0.03	1.00±0.10^{b}	1.63±0.12^{a}	1.27±0.14^{ab}	1.00±0.14^{b}	1.70±0.16^{a}	1.63±0.16^{a}
IL-2	1.00±0.12	1.23±0.39	1.27±0.04	1.00±0.31^{b}	2.25±0.42^{a}	1.25±0.24^{b}	1.00±0.18	3.74±1.84	1.08±0.15
IL-6	1.00±0.10^{b}	1.10±0.10^{b}	1.63±0.15^{a}	1.00±0.07	1.00±0.08	1.30±0.08	1.00±0.14	0.99±0.05	1.21±0.13
IL-10	1.00±0.17	0.99±0.25	1.47±0.12	1.00±0.24	1.42±0.18	0.94±0.07	1.00±0.05	2.11±0.44	1.58±0.16
IL-1β	1.00±0.06^{b}	1.59±0.79^{ab}	2.56±0.25^{a}	1.00±0.25	1.76±0.31	1.48±0.10	1.00±0.15^{b}	3.35±0.37^{a}	3.04±0.40^{a}
IFN-α	1.02±0.12	1.15±0.36	1.19±0.04	1.00±0.17^{b}	1.41±0.28^{ab}	1.54±0.12^{a}	1.00±0.19	1.13±0.31	0.87±0.10
Occludin	1.00±0.08^{b}	1.23±0.25^{b}	2.30±0.21^{a}	1.00±0.06^{b}	1.98±0.17^{a}	1.32±0.10^{b}	1.00±0.12	1.44±0.08	1.24±0.07
TLR-4	1.00±0.06^{b}	1.10±0.24^{b}	2.42±0.62^{a}	1.00±0.15^{b}	1.91±0.24^{a}	1.35±0.16^{ab}	1.00±0.14	1.56±0.17	1.57±0.12
TNF-α	1.00±0.13	1.16±0.41	1.97±0.22	1.00±0.04^{b}	2.09±0.64^{a}	2.25±0.30^{a}	1.00±0.05	2.48±0.24	2.54±0.30
ZO-1	1.00±0.08	1.22±0.13	1.95±0.29	1.00±0.02	1.54±0.20	1.06±0.09	1.00±0.14	1.31±0.18	1.09±0.09

Data in the same row with different superscripts differ significantly ($P < 0.05$). IL, interleukin; IFNα, Interferon-alpha; TLR, toll-like receptor; TNFα, tumor necrosis factor alpha; ZO-1, zonula occludens-1. The same below.

Figures

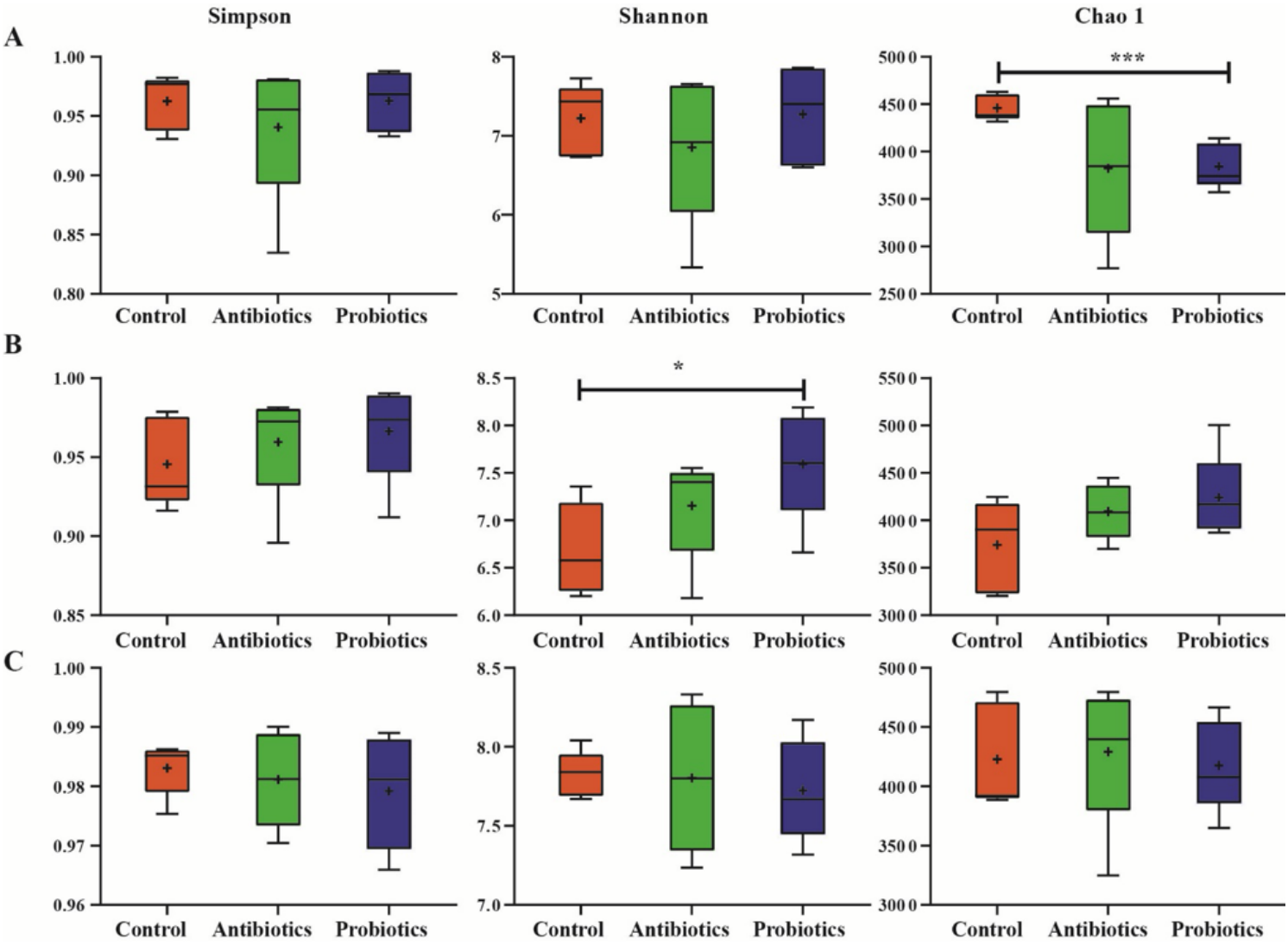

Figure 2

Alpha diversity of the colonic bacterial community of weaned piglets with different treatments. A, B, and C means Day 7, Day 21, and Day 42 of trial, respectively. Control: a blank control group (basal diet); Antibiotics: a antibiotic group (supplemented with 0.04 kg/t virginiamycin, 0.2 kg/t colistin, and 3000 mg/kg zinc oxide); Probiotics: a probiotics group (supplemented with 4×109 CFU/g BLS mix). The same below.

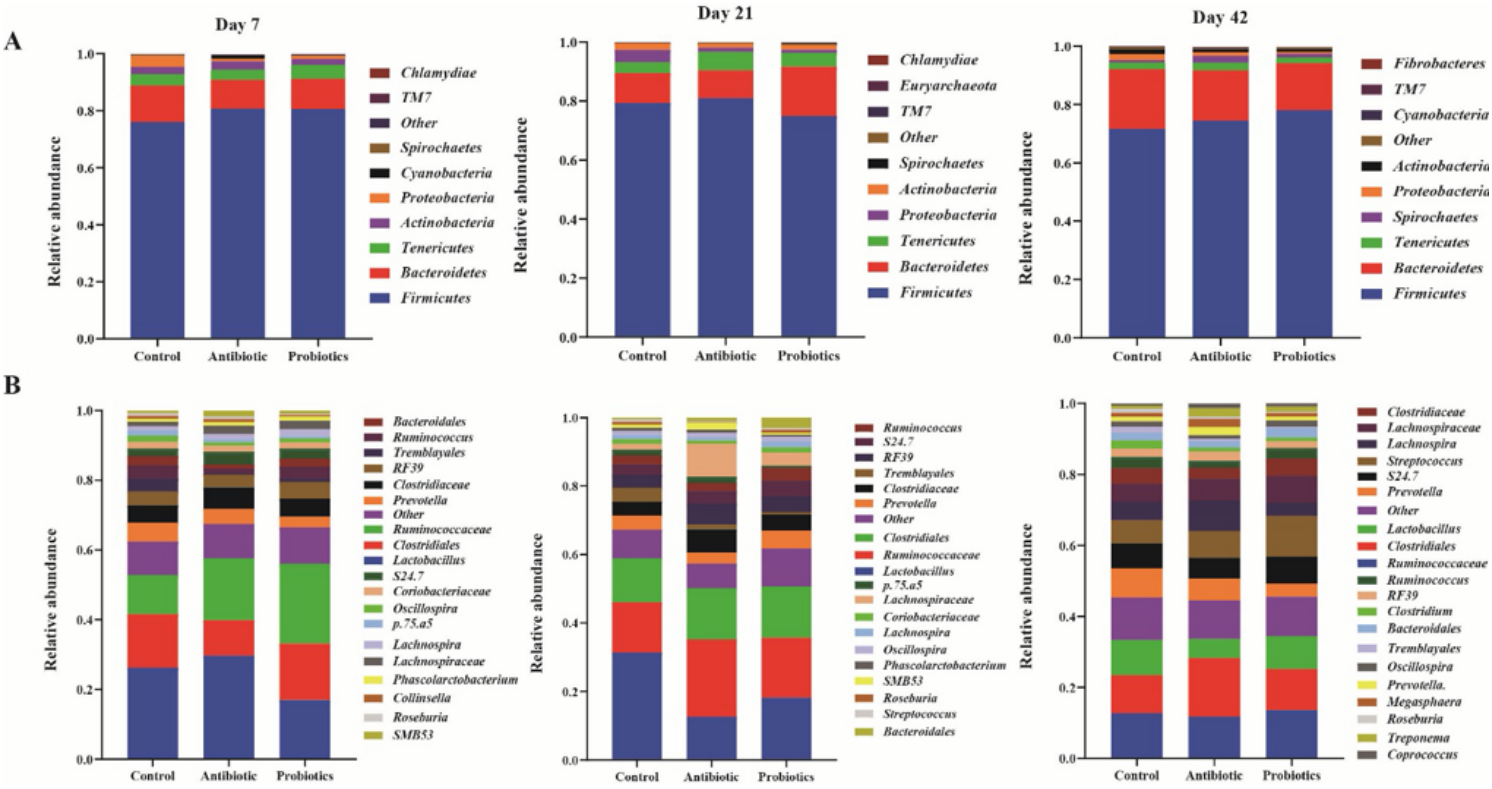

Figure 4

Colonic microbiota composition of weaned piglets with different treatments. Microbial community bar plot at the phylum level (A) and genus level (B).

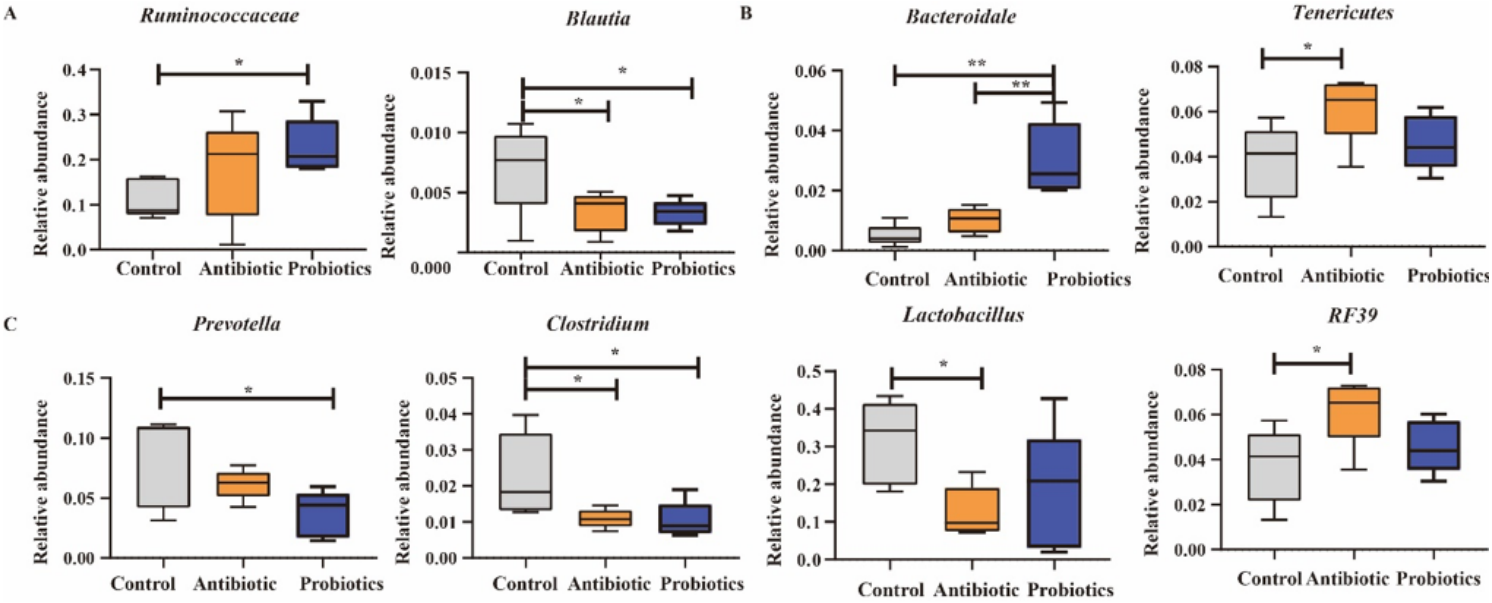

Figure 6

Comparison of the colonic microbial community in weaned piglets from control, antibiotic, and probiotics groups. Values are expressed as means ± SE. *P < 0.05, and **P < 0.01. (A), (B), and (C) means Day 7, Day 21, and Day 42, respectively. The same below.

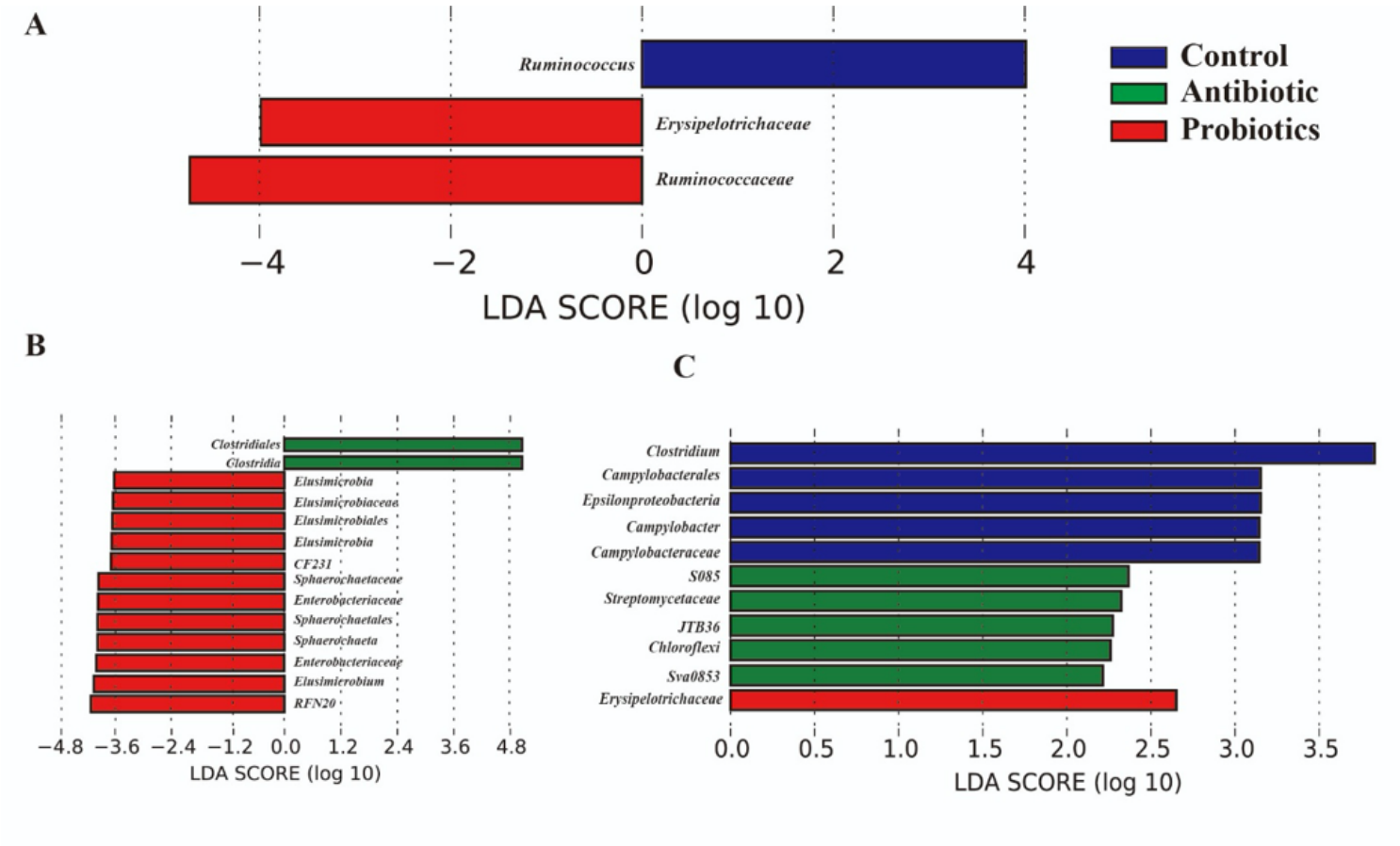

Figure 8

LefSe analysis of the colonic microbial community in weaned piglets from control, antibiotic, and probiotics groups

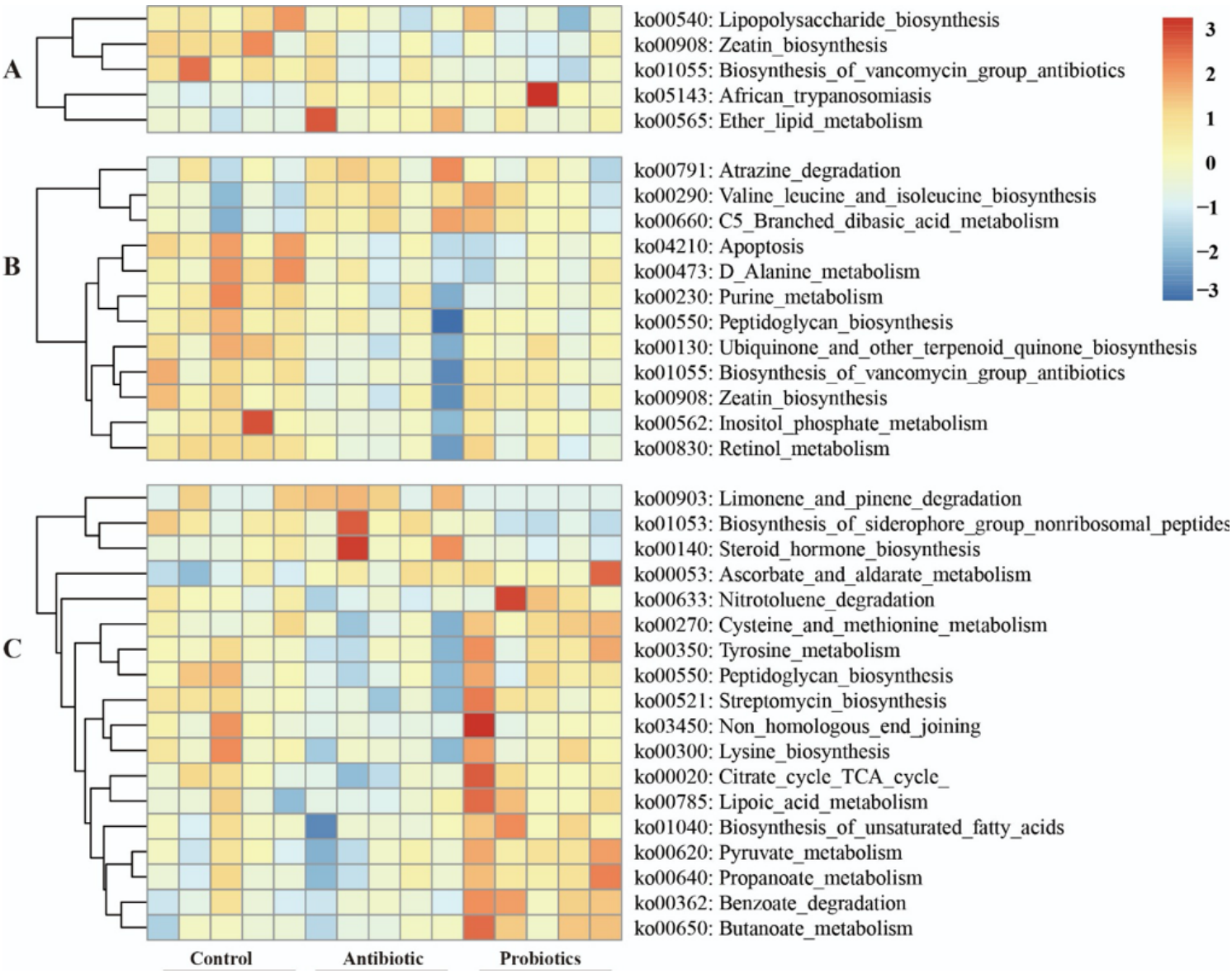

Figure 10

PICRUSt and KEGG analysis of the colonic microbial community in weaned piglets from control, antibiotic, and probiotics groups

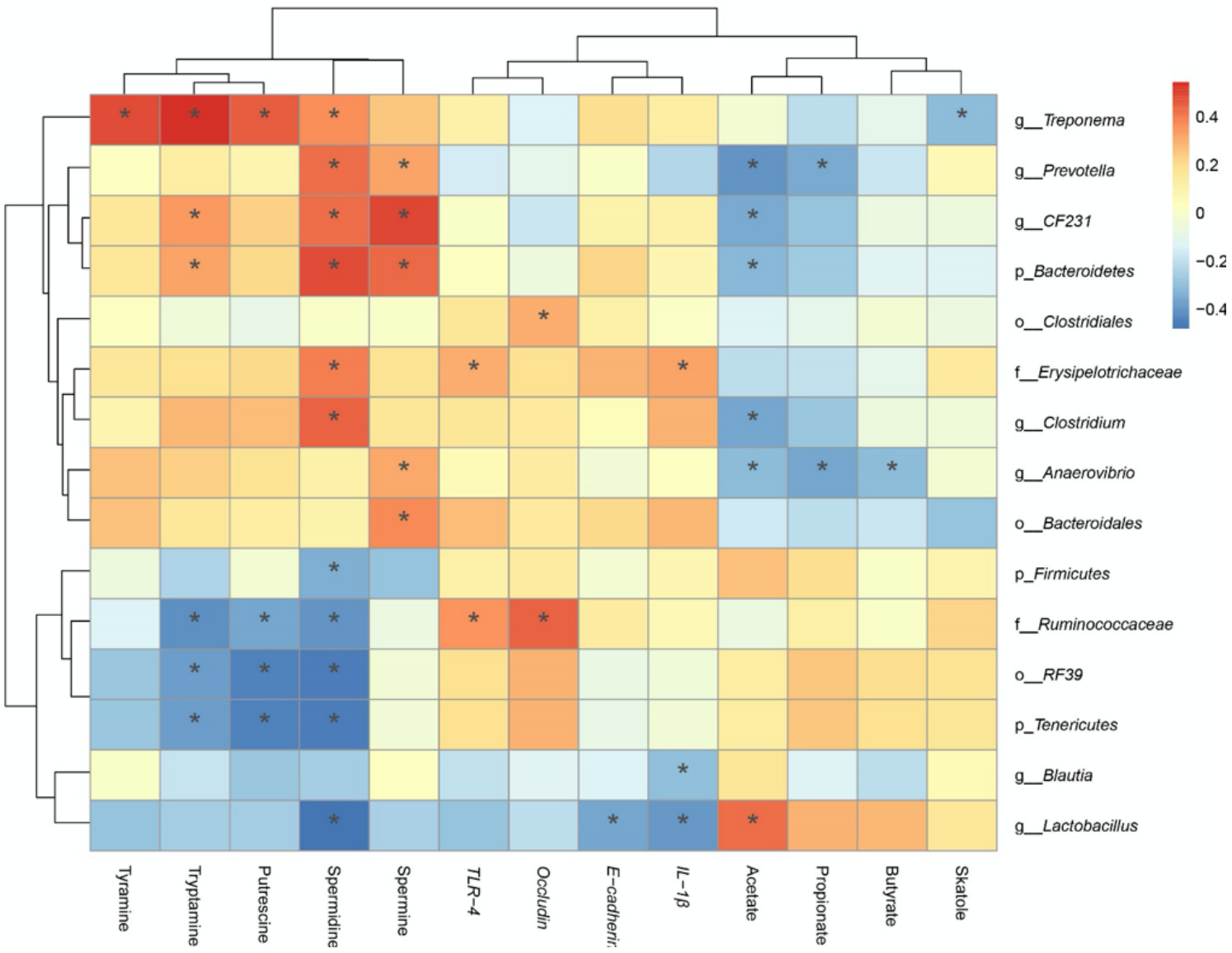

Figure 12

Correlations between the microbiota, health parameters, and colonic metabolite concentrations in weaned piglets

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