Administration of *Saccharomyces boulardii* mafic-1701 improves growth performance, promotes antioxidant capacity, alleviates intestinal inflammation and modulates gut microbiota in weaned piglets

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Research

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Abstract

Background: Probiotics seem to be an alternative to antibiotics for improving animal's health and intestinal development. Saccharomyces boulardii (S. boulardii) is a well-known probiotic. However, only few studies have been performed examining the effects of S. boulardii on weaned piglets . Therefore, this study was conducted to investigate the effects of dietary S. boulardii mafic-1701 on growth performance, antioxidant parameters, inflammation and intestinal microbiota in weaned piglets, using aureomycin as positive control. One hundred and eight piglets were randomly divided into three dietary treatment groups: (1) basal diet (CON); (2) basal diet supplemented with 75 mg/kg aureomycin (ANT); (3) basal diet supplemented with 1 × 10 8 CFU/kg S. boulardii mafic-1701 (SB).

Results: Compared to CON, the supplementation with S. boulardii mafic-1701 improved feed efficiency over the entire 28 days ($P < 0.01$) and decreased the rate of diarrhea during the first week ($P < 0.05$). Total superoxide dismutase concentration was markedly increased in piglets with S. boulardii mafic-1701 ($P < 0.01$). Moreover, compared with CON, SB increased the concentration of interleukin-4 in ileum ($P < 0.05$), while the level of pro-inflammatory cytokines interleukin-6 ($P < 0.01$) and tumor necrosis factor ($P < 0.01$) were decreased in jejunum. SB increased the abundance of Bacillus and Ruminococcaceae ($P < 0.05$), whereas the population of Clostridiaceae were decreased ($P < 0.05$). Furthermore, the analysis of microbiota metabolites showed that S. boulardii mafic-1701 administration increased the concentration of formate and isobutyrate in cecum to maintain a stable microbiota and gut health ($P < 0.05$).

Conclusion: This study indicated that S. boulardii mafic-1701 supplementation could improve growth performance, alleviate the severity of diarrhea in weaned piglets, which may be associated with S. boulardii mafic-1701 promoted antioxidant activity, anti-inflammatory responses and microbi al ecology of piglets.

Background

In order to shorten the slaughter cycle of pigs and get sows reproductive better, the early-weaning strategy has been applied in commercial pig production [1], with weaning age has been decreasing [2]. It is one of the most stressful matters in pig’s life [3]. To relieve weaning stress, traditional antibiotics have been used markedly in recent decade. While, considering the intensifying substantial environmental pollution, arise antimicrobial resistance and global public health crisis, several replacements have been developed over the past years, including antimicrobial peptides, prebiotics, new antibiotics, anti-virulence molecules, antibodies and probiotics [4].

Probiotics are defined as “friendly” live microorganisms and when administered probiotics in

adequate amount they will confer a health benefit on host [5]. For many years since their introduction, the major use has been focused on animal feeds [6], the most commonly used probiotic microorganisms are bacteria, for instance *Lactobacillus* and *Bifidobacterium*. Species from other bacterial genera such as *Enterococcus*, *Streptococcus* and *Bacillus* are also widely used as probiotic [7]. The most usual

application and the advantageous effects of probiotics should be associated with some widespread mechanisms include: 1) Colonization resistance, 2) Normalization of perturbed microbiota, 3) Acid and short chain fatty acids (SCFAs) production, 4) Increased turnover of enterocytes, 5) Regulation of intestinal transit, 6) Competitive exclusion of pathogens [5]. This is also true for the yeast probiotic, namely, *Saccharomyces boulardii* (*S. boulardii*). *S. boulardii* is one of the most commonly employed probiotics in recent years, which is a safe, efficacious and non-pathogenic yeast isolated from lychee fruit in Indochina and it belongs to the group of species *Saccharomyces cerevisiae* (*S. cerevisiae*) [8, 9]. Previous study found that *S. cerevisiae* have probiotic capacity [10]. However, *S. boulardii* possesses a superior probiotic efficiency than *S. cerevisiae* because *S. boulardii* exhibits several distinct physiological and metabolic characteristics considerably different from *S. cerevisiae* [8]. Notably, *S. boulardii* could tolerant and growth at high temperature of 37 ℃. It's a unique advantage of *S. boulardii* that do best at piglets. Moreover, *S. boulardii* has a great resistance to gastric acidity, bile and proteolysis [11, 12]. As it good at tolerating vary pH levels and enzymes, it could better survival in the intestinal surface than that of bacterial probiotics. *S. boulardii* is naturally resistant to antibiotics, so it can be prescribed during antibiotic treatment. In addition, it is the only yeast strain that is described as a probiotic against gastrointestinal diseases [13]. As we expected, accumulating evidence suggested that oral administration *S. boulardii* may be protected against antibiotic associated diarrhea and improved *clostridium difficile* (*C. difficile*) associated colitis in animal experiments [8, 14]. In human studies, administration *S. boulardii* protected *C. difficile* infection, mitigated intestinal microbiota disorder and reduced antibiotic associated diarrhea [15–17].

The beneficial properties mentioned here would indicate *S. boulardii* is a promising probiotic as feed additive in animal production. However, intervention studies and investigations of long term *S. boulardii* effects on weaned piglets remains less clear. Therefore, the objective of this study was to determine the hypothesis, if *S. boulardii* mafic-1701 supplementation to diets will promote growth performance, antioxidant capacity in serum, gut anti-inflammatory responses, microbiota composition and fermentation metabolites products in weaned piglets.

Materials And Methods

Experimental protocols of this study include piglets handling and treatment were approved by the "Institutional Animal Care and Use Committee of China Agricultural University" (ICS 65.020.30). All animal procedures were carried out in accordance with the specifications of the National Research Council's Guide for the Welfare and Ethics of Laboratory Animals.

Probiotic strain and culture conditions

The yeast *S. boulardii* mafic-1701 was isolated by our laboratory and kept on Yeast Extract Peptone Dextrose (YPD) agar plates to screen single colonies. Colonies of *S. boulardi* mafic-1701were inoculated in YPD medium for 16 h at 37 ℃ to prepare seed cultures. High density fermentation cultivation was performed using a fermentor (30 L) with an initial volume of 15 L of medium with the following

composition (g/L): dextrose, 50; corn steep liquor powder, 25; $(NH_4)_2SO_4$, 4; KH_2PO_4, 2; $MgSO_4$, 0.5. 750 mL of seed cultures were added into medium. The initial dissolved oxygen concentration was adjusted to 30%. The pH was set at 6.5 using 3 M NaOH. Fermentation was processed at 37 ℃ at 250 rpm with an aeration rate of 5 L/min of air. The pH was maintained at 6.5 by the addition of 3 M NaOH and anti-foaming agents was automatically added when each time foam was generated. To measure the biomass of *S. boulardii* mafic-1701 fermented samples were collected every 12 h. The actual yeast product used in this present study was obtained by mixing the precipitated of fermentation broth with 21.57 kg wheat brans and the product moisture content finally controlled at 2% by heat drying.

Experimental design and diets

A randomized controlled experiment was undertaken on Feng Ning Swine Research Unit of China Agriculture University (Academician Workstation in Chengdejiuyun Agricultural & Livestock Co., Ltd). A total of 108 piglets (Duroc × (Landrace × Yorkshire), mean body weight: 8.5 ± 1.1 kg) were randomly assigned to three dietary treatment groups, based on their gender and initial body weight. Each treatment involved 6 replicates and each replicate consisted 6 piglets. All piglets were housed in identical conditions and they had ad libitum to access water and food. Basal diets (Table 1) in this study were formulated to meet or exceed the nutritional requirements of sucking piglets at each stage of growth as recommended by the NRC (2012). Dietary treatment groups consisted of basal diet (CON), basal diet supplemented with 75 mg/kg aureomycin (ANT) and basal diet supplemented with 1×10^8 CFU/kg *S. boulardii* mafic-1701 (SB).

Performance and diarrhea incidence

Piglets were weighted and recorded individually on days 0, 14 and 28 while feed consumption of each pen were determined on days 0, 14 and 28. The average daily gain (ADG), average daily feed intake (ADFI) and feed to gain ratio (F:G) were calculated by pens. To evaluate the rate of diarrhea, fecal consistency was visually assessed three times per day by fixed observers blind to the treatment according to the method described by Hart and Dobb [18]. The scoring system was applied to determine the rate of diarrhea as following: 1 = normal feces; 2 = possible slight diarrhea; 3 = fluid feces; 4 = very watery diarrhea. The occurrence of diarrhea was defined as maintaining fecal scores of 3 or 4 for 2 consecutive days. The rate of diarrhea was calculated according to the following formula: the rate of diarrhea (%) = (number of piglets with diarrhea × diarrhea days)/(number of piglets × total observational days) × 100 [19].

Sample collection and processing

One piglet was selected from each pen with the intermediate body weight and piglets were slaughtered on day 28 of the experiment. Prior to slaughter, 7 ml blood samples were collected from jugular vein using vacuum blood tube without anticoagulant. Samples of blood were centrifuged at 3,000× g for 15 min. Serum samples were separated and stored at -20 ℃ for further analysis. Various subsamples from cecum and colon digesta were immediately taken and stored at liquid nitrogen for different analysis. One

aliquot of digesta samples were obtained for microbial composition analysis and additional subsamples were taken to determine the bacterial metabolites in the gut. Intestinal samples from jejunum and ileum without gut contents were taken and kept at -80 ℃ for anti-inflammatory analysis.

Determination of serum immune parameters and antioxidant indexes

The concentration of serum immune indices including IgA and IgG were detected according to protocols provided by the commercially available piglet serum ELISA kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Assessment of antioxidant parameters were performed by determination the concentration of total superoxide dismutase (T-SOD), malondialdehyde (MDA), total antioxidant capacity (T-AOC) and glutathione Peroxidase (GSH-P_X) in the serum using commercially available piglet serum ELISA kits according to instructions described by the manufacturer (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Cytokine measurement

The concentration of interleukin-8 (IL-8), interleukin-4 (IL-4), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in jejunum and ileum were determined by the commercially available piglet ELISA kits according to instructions of the manufacturer (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Microbiota analysis

Microbial community genomic DNA was isolated from cecum and colon digesta samples, using the E.Z.N.A.® stool DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to the manufacturer's methods. The V3-V4 regions of the bacterial 16S rRNA gene were amplified by PCR using universal primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') with following procedures: initial denaturation at 95 ℃ for 3 min, followed by 27 cycles of denaturing at 95 ℃ for 30 s, annealing at 55 ℃ for 30 s, extension at 72 ℃ for 45 s, single extension at 72 ℃ for 10 min and end at 4 ℃. Illumina sequencing was performed, the raw data were quality-filtered by Trimmomatic and merged by FLASH software with the following criteria: (i) the average quality score less than 20 were truncated at any site. 50 bp sliding window was set and the reads, which shorter than 50 bp or containing ambiguous were discarded; (ii) sequences with longer than 10 bp were assembled in light of their overlapped sequence. The maximum mismatch ratio of overlap area is 0.2. Unassembled reads were discarded; (iii) Samples were noticed and understood according to their barcode and primers besides the sequence was adjusted to a right direction.

Using UPARSE (version 7.1, http://drive5.com/uparse/) operational taxonomic units (OTUs) with 97% similarity cutoff were clustered and chimeric sequences were filtered out. Each 16S rRNA representative gene sequence was categorized and analyzed by RDP Classifier (http://rdp.cme.msu.edu/) against the Silva (SSU128) 16S rRNA database using confidence threshold of 70%.

Quantification of fermentation products

Approximately 0.5 g of intestinal digesta was weighed into a 10 mL tube and diluted 1:16 with ultrapure water (8 mL). Glass spheres were added and vortexed to homogenize the contents. After ultrasonic wave, samples were centrifuged at 4000 r/min for 15 min. One hundred and sixty microliters supernatant was transferred into a 10 mL tube with 7.84 mL ultrapure water then filtered through a 0.22-μm filter. Finally, the concentrations of SCFAs in extracted sample solution were determined by high-performance ion chromatography.

Statistical analysis

Data on growth performance, serum immune and antioxidant indices, inflammatory cytokines and SCFAs were analyzed by one-way ANOVA using SPASS19.0 software (SPSS Inc., Chicago, IL, USA). Least-significant difference (LSD) tests were used to determine the differences between CON, ANT and SB. Treatment effects were considered statistical significance if P less than 0.05.

Results

Diarrhea incidence and Growth performance

In commercial pig production, piglets are abrupt separated from the sows and characterized by stressful changes such as diarrhea. The first step of our study was to investigate whether *S. boulardii* mafic-1701 administered could relieve the diarrhea of piglets (Fig. 1). During the first week, SB markedly decreased the diarrhea incidence compared to CON ($P < 0.05$). Similarly, ANT also attenuated the diarrhea incidence ($P < 0.05$) compared with CON. Whereas, no significant difference in diarrhea incidence was observed between SB and ANT ($P > 0.05$). During the last week as well as over the entire four weeks, there were no significant differences in the rate of diarrhea among three treatment groups. Growth performance data (Table 2) were shown that there were no significant changes in ADFI and ADG among three treatment groups ($P > 0.05$). During d 0 to 14 and d 15 to 28 of the experiment, Compared with CON, SB showed a decreased F:G ($P < 0.05$). In the whole experiment period, administration of aureomycin and *S. boulardii* mafic-1701 to the piglets significantly reduced F:G compared with CON ($P < 0.01$).

Serum immune and antioxidant parameters

The concentration of IgA and IgG among three treatment groups were analyzed, we found that oral aureomycin and *S. boulardii* mafic-1701 did not significantly impact serum immune indices compared with oral basal diet (Table 3). The concentration of T-SOD was increased in the serum of piglets with SB compared to piglets with CON ($P < 0.01$), whereas piglets with or without antibiotic maintained similar results ($P > 0.05$). For the concentration of MDA, relative to CON, SB was markedly lower in the serum of piglets ($P < 0.05$). In addition, oral aureomycin and *S. boulardii* mafic-1701 did not significantly effect on the concentration of T-AOC and GSH-P_X in the serum (Table 3).

Effect of *S. boulardii* mafic-1701 administration on intestinal inflammatory responses

Indices related to gut inflammation were evaluated in intestinal tissues including jejunum and ileum (Table 4). In this study, the concentration of TNF-α and IL-6 in the jejunum decreased significantly in ANT and SB compared to CON ($P < 0.01$). In contrast the concentration of TNF-α and IL-6 in the ileum did not differ among three treatment groups. As shown by Table 4 the secretion of IL-8 in the jejunum decreased significantly only in ANT compared with CON ($P < 0.05$). While the concentration of IL-8 was decreased in both ANT and SB compared with CON in the ileum ($P < 0.05$). Moreover, the results showed that differences on the concentration of IL-4 in the jejunum were not observed among three treatment groups ($P > 0.05$). However, supplementation with a single dose of *S. boulardii* mafic-1701 was significantly increased the production of IL-4 compared with CON in the ileum ($P < 0.05$).

Intestinal microbiota composition

The OTUs were classified for bacteria community on the basis of usable sequence at a 97% similarity level. The analysis of OTUs were primary shown in Fig. 2 corresponding to cecum and colon digesta samples of three treatment groups including CON, ANT and SB, there were 42, 66, 268 core OTUs specifically each of these three individual groups, respectively and a total of 325 core OTUs were common to all treatment groups. Total 712 OTUs were shared in colon digesta samples among three treatment groups with 419, 318, 799 OTUs confined to CON, ANT and SB, respectively. Expect that, the vast majority of ANT core OTUs were shared with SB OTUs but only 152 core OTUs that were shared between CON and SB and only 138 OTUs were shared between CON and ANT. These results indicated that ANT and SB were more similar in terms of OUT analysis. Fig. 3 depicted the microbial composition of cecum and colon digesta samples across three treatment groups. At phylum level, 6 phyla in cecum digesta samples were indicated that *Firmicutes* was the most predominant phyla of bacteria among three treatment groups. Moreover, *Bacteroidetes* was the second abundant phyla in ANT and SB. Fig. 3 also showed that *Firmicutes* and *Proteobacteria* were dominating bacteria in the colon digesta samples of weaned piglets. Principal component analysis (PCA) based on Bray-Curtis distances was measured in cecum and colon microbiota (Fig. 4). PCA analysis was conducted, the results indicated that SB was obviously separated in comparison to CON and ANT in cecum microbiota. The PCA analysis of microbiota from colon digesta samples showed that CON was relatively distinct compared to those in ANT and SB. In contrast, we found that the colon microbiota of ANT and SB was more similar. The differences in the relative abundance of microbiota in cecum and colon digesta samples among three treatment groups were shown in cladograms, and the linear discriminant analysis (LDA) scores greater than or equal to 2.0 were confirmed by the linear discriminant analysis effect size (LEfSe). In cecum digesta samples (Fig. 5), the proportion from the *Bacillaceae* family to *Bacillales* order was significantly increased in SB ($P < 0.05$). Moreover, *Ruminococcaceae* at genus level was enriched in SB ($P < 0.05$). In colon digesta samples (Fig. 6), the proportion of *Bacillus* at the genus level was significantly increased by combined with *S. boulardii* mafic-1701 ($P < 0.05$), while greater relative abundance of *Lactobacillales* order and *Prevotella* genus were observed in CON ($P < 0.05$). In addition, the abundance of *Clostridiaceae* family and *Clostridiaceae* genus was significantly enriched in ANT ($P < 0.05$).

Concentrations of fermentation metabolites products

SCFAs in the cecum and colon digesta were presented in Table 5. There were no significant differences by the experimental treatments on the cecum digesta including acetate, butyrate, and propionate ($P > 0.05$). However, a significant increase in the concentration of colonic propionate in ANT compared with CON ($P < 0.01$). This pattern was also found on acetate and butyrate in the colon digesta with numerical differences ($P < 0.05$). Furthermore, we found no significant differences for colonic formate among three treatment groups ($P > 0.05$) but the level of cecal formate was significantly increased in SB compared with CON ($P < 0.05$). In the cecum digesta, the aureomycin as well as *S. boulardii* mafic-1701 administration significantly increased the concentration of valerate compared with CON ($P < 0.05$). Additionally, only the concentration of isobutyrate in SB significantly increased more than CON and ANT in the cecum digesta ($P < 0.05$).

Discussion

S. boulardii is an important microorganism, which has been the most frequently used as a safe probiotic additive in weaning piglets. Weaning is a critical period in the pig production cycle with a sudden, stressful, short and complex event due to the change in diet and a new living environment conditions [2], leading to reduce feed intake, increase the risk of environment result in gut inflammation, which affect morphological and function of intestinal, humoral immunity in the gut, tissue damage, intestinal microbial ecosystem and increase the incidence of diarrhea [20]. Previous study demonstrated more than two weeks must be required for weaning piglets to adapt to these changes. Therefore, in this study we investigated the effects of dietary *S. boulardii* mafic-1701 supplemented in the diet on piglet health and gut microbiota composition in a long term intervention.

In the present study, ADFI and ADG were not changed in the whole feeding trials. However, the result showed that weaned piglets supplemented with *S. boulardii* mafic-1701 significantly improved feed efficiency. In addition, as a positive control, in the whole period of the experiment, supplement aureomycin with the diet had significantly decreased F:G. these results suggested that the inclusion of *S. boulardii* mafic-1701 in diet could maintain similar feed efficiency as piglets fed the antibiotic diet.

Weaning piglets in CON exhibited diarrhea with semi-liquid or watery faces. During 0–14 days, compared with CON, ANT and SB were significantly decreased the rate of diarrhea. Interestingly, on days 15–28 and the overall experiment period did not markedly differ across three treatment groups. Obviously, diarrhea of piglets was not caused by the use of the antibiotics, in other word it was not antibiotic associated diarrhea. Thus it can be speculated that the diarrhea was relevant to post-weaning stress, although the exact underlying mechanisms remain unclear [21]. However, oral administration aureomycin or *S. boulardii* mafic-1701 could minimize the adverse effects of weaning stress during the first week, but the incidence and frequency of diarrhea was all decline and did not differ among three treatment groups during the whole 28 days. These results suggested that the piglets may be adapt to the new social and physical environment regardless of the diets.

It is generally known that the deleterious effects of weaning on the piglets gastrointestinal are vast, although the mechanisms by weaning influences intestinal function are less clear, it is certain that weaning could lead to drastic changes in breakdown of intestinal barrier functions [22]. The intestinal barrier is composed of intestinal epithelium cells and the tight junction proteins of epithelial cells [4]. The intestinal epithelium has a prominent and visible capacity for defensing against potentially harmful microorganisms as the first physical barrier line [23]. When the intestinal barrier is breakdown, it can result in pathogen microbiome colonization and takes with the risk of inflammation [24]. It is associated with the activation of innate immunity and inflammatory responses. In this study, we found that the concentration of pro-inflammatory cytokines such as TNF-α, IL-8 and IL-6 were decreased in ANT and SB compared with CON. We have also found that the production of IL-4 was increased by oral administration *S. boulardii* mafic-1701. These results indicated that *S. boulardii* mafic-1701 has beneficial effects on intestinal inflammation. Similarly, it has been previously shown that *S. boulardii* administration was significantly decreased mRNA expression of cytokines including interleukin-1β and interleukin-12 [25]. nuclear factor kappa B (NF-*κ*B) is a pivotal regulator of innate immune responses in the gut [8]. Previous study has shown that *S. boulardii* blocked NF-*κ*B activation and reduced colonic inflammation [26]. Thus, we can assume that *S. boulardii* mafic-1701 could alter the concentrate of pro-inflammatory cytokines through modulate the signal pathway implicated in pro-inflammatory responses such as inhibit the NF-*κ*B associated pathways activation. Nevertheless, we do not exclude that exit other mechanisms could account for modulating effect of *S. boulardii* mafic-1701 on inflammatory responses. However, there is no doubt that *S. boulardii* mafic-1701 possess an anti-inflammatory property in the gut.

Previous study demonstrated that probiotics could activate the local mucosal protective mechanisms and exert beneficial effects on the host such as modulate anti-oxidation and immune responses [27]. In our study, we observed that *S. boulardii* mafic-1701 and aureomycin supplementation had no effect on increasing the levels of IgA and IgG in the serum. It was inconsistent with the previous observation by JEAN-PAUL, who reported that secretory IgA and secretory constituent of immunoglobulins in the rat small intestinal was increased by treated with *S. boulardii* [28]. Indeed, different probiotic strain could exert different physiological effects, therefore the differential immune response of piglets may be related to the type and does of probiotic strains we used. Besides, it is also associated with the animal models we chose to use in this study. In the future, the verdict need to be validated. Furthermore, in terms of antioxidant analysis. In our study, we found that T-SOD was increased in SB in the serum of the piglets, which suggested *S. boulardii* mafic-1701 plays a critical role to improve antioxidant capacity and protect intestinal mucosa [27].

The greatest and most diverse cluster of microorganisms was inhabited in the gut [29]. The gut microbiota has symbiotic relationship with the host, which provides an ideal survival environment for microbes while the microbes provide a broad range of functions for the host such as defense against pathogens, digestion of complicated dietary nutrients, production of beneficial metabolisms and maintenance of the immune system [29]. Diet can drive gut microbiota composition, for instance, Antibiotic treatment can alter the balance of compositional in the intestinal microbiota. In contrast, oral ingestion of probiotics can enhance this delicate balance between host and gut microbes. In fact, the core

advantage of the probiotic is considered by supporting a stable immune system. Oral administration probiotics can improve gut health by stimulating the growth of some beneficial microbes and inhibiting pathogens invasion [30]. In present study, PCA indicated that diet is the most crucial factor contribute to changes in microbiota structure, considering that groups were matched for age, had no difference in environment and received no recent medication. Compared with the other regimens, oral administration of *S. boulardii* mafic-1701 was showed that the largest variety in microbiota in the cecum and colon samples. Namely, the composition and structure of the microbiota was greatly affected by diet. From the results of phylum analysis, we found that the cecum microbial floras were dominated by *Firmicutes*, this was consistent with previous finding reported by Lei Yu [31]. Whereas colon microbiota communities were dominated by *Firmicutes* and *Proteobacteria*, regardless of diet treatment. The colonization of *Proteobacteria* was increased in the colon that was expected because the members of *proteobacteria* are facultative anaerobes [32] and the colon is a completely anaerobic environment which could provide a favorable living environment for them [33]. From the results, we found higher bacterial diversity in cecum and colon of the piglets fed with *S. boulardii* mafic-1701 in the feeding trial. In cecum, one of the microbiota enriched in SB was *Ruminococcaceae*. In other word, the population of *Ruminococcaceae* genus was increased by dietary *S. boulardii* mafic-1701 administration. This should be associated with *S. boulardii* cell wall composition, the yeast cell wall consists of Mannose, chitin, 1,3-β- glucan and 1,6-β-glucan [8]. While *Ruminococcaceae* species may be have a wonderful ability to utilize simple and complex sugars and polysaccharides then the production of a certain amount of volatile fatty acids as an energy sources which can be utilized by the host [34].The result investigated that *S. boulardii* mafic-1701 inclusion had beneficial effects on giving a balanced gut microbiota with high stability in a long term intervention.

In the colon, *S. boulardii* mafic-1701 inclusion showed some alterations in regard to microbiota communities in weaned piglets. *Bacillus* is identified as a helpful microbe to modulate gut health, they can germinate to form metabolically active and with the ability to survive hostile environments [35]. Several species of *Bacillus* can reduce dextran sulfate sodium induced colitis and improve the production of SCFAs [36]. In this study, *S. boulardii* mafic-1701 inclusion increased the abundance of *Bacillus* genus. Previous study reported that several *Bacillus* species, which are considered to reduce pathogen colonization by unclear mechanisms [35]. Notably, the relative abundance of *Clostridiaceae* family was significantly increased in ANT compared with SB. *C. difficile* belongs to *Clostridiaceae* and is generally regarded as the most major etiologic factor for antibiotic associated diarrhea and colitis, *C. difficile* mediates intestinal disorder by releasing two potent exotoxins, toxin A and toxin B [37]. The diagnosis was further confirmed that antibiotic treatment altered the composition of the gastrointestinal microbiota, cause the gut dysbiosis that manifest the host susceptible to pathogen infection and trigger the immunological dysregulation [38]. In contrast, *S. boulardii* mafic-1701represents one of the most effective probiotic that have ability to treat antibiotic associated diarrhea and prevent pathogen infection [37]. *Lactobacillus* belongs to the phylum *Firmicutes*, class *Bacilli*, order *Lactobacillales* and family *Lactobacillus* [39], which is characterized by antibacterial and anti-inflammation activities. Previous study has reported that *Lactobacillus* spp. are beneficial to the host due to their underlying effects on gut

function and health [40]. Nevertheless, the present study showed that the relative abundance of *Lactobacillus* genus was decreased in SB compared with CON. The reason for this result might be associated with the increased diversity of the microbiota induced by inclusion of *S. boulardii* mafic-1701 in diet [41].Moreover, the community of *Lactobacillus* in porcine intestinal would decreased in time [42]. *Prevotella* strains are negatively linked with chronic inflammatory conditions[43]. As we expected, a reduction on the population of *Prevotella* was observed in piglet supplied *S. boulardii* mafic-1701. Taken together, these observations further point to the beneficial effect of *S. boulardii* mafic-1701 on regulating the gut microbial ecology and *S. boulardii* mafic-1701 may be contribute to a lifetime health of piglets.

The intestinal microbiota is a signaling hub that integrates diet [44], meanwhile, diet drives intestinal microbiota composition and metabolism. A growing body work demonstrated that microbial produced metabolites as crucial executors to regulate the health on the host [29]. SCFAs play a central role in gut metabolism [45]. A previously published report indicated that probiotics can enhance SCFAs production [46]. In this study, we found that the production of valerate was significantly increased in ANT and SB compared with CON in the cecum. The finding demonstrated that *S. boulardii* mafic-1701 could enhance SCFAs levels and provided an evidence that *S. boulardii* mafic-1701 can substitution antibiotics to a certain extent. In addition, previous study showed that the major metabolites from the microbial fermentative activity are acetate, propionate, and butyrate [29]. In the present study, it's worth noting that the production of colonic acetate, propionate and butyrate were significantly increased only in ANT, which might be associated with a number of factors, for instance, an increase in dietary intake is the most important element [46] and this hypothesis is accord with the results of growth performance which we investigated. And beyond that, the principal site of fermentation is proximal colon thus the production of SCFAs are depended on the numbers and the types of microbes which colonized in the colon [46].

Conclusion

In conclusion, weaner diet containing *S. boulardii* mafic-1701 promoted the growth performance, alleviated the severity of diarrhea, improved antioxidant activity and anti-inflammatory responses in weaned piglets. The diversity of intestinal microbiota and their fermentation products were increased via a long-term *S. boulardii* mafic-1701 intervention. In particular, *Ruminococcaceae* spp. and *Bacillus* spp. were distinct bacteria with higher abundances. These changes should facilitate the maturation of the digestive system of piglets in the subsequent growing phases.

Abbreviations

S. boulardii: *Saccharomyces boulardii*; SCFAs: Short chain fatty acids; *S. cerevisiae*：*Saccharomyces cerevisiae*; *C. difficile*: *Clostridium difficile*; YPD: Yeast Extract Peptone Dextrose; ADG: Average daily gain; ADFI: Average daily feed intake; F:G: Feed to gain ratio; T-SOD: Total superoxide dismutase; MDA: Malondialdehyde; T-AOC: Total antioxidant capacity; GSH-P_X: Glutathione Peroxidase; IL-8: Interleukin-8; IL-4: Interleukin-4; IL-6: Interleukin-6; TNF-α: Tumor necrosis factor-α; OTUs: Operational taxonomic units;

PCA: Principal component analysis; LDA: The linear discriminant analysis; LEfSe: The linear discriminant analysis effect size; NF-κB: Nuclear factor kappa B.

Declarations

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Not applicable.

Author's contributions

WXZ and YHC designed the experiment. WXZ, CLB and JW performed the experiment. JJZ supervised the whole experiment. WXZ wrote the paper, YHC edited the paper. All authors read and approved the final manuscript.

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Availability of data and materials

The data analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by Committee of China Agricultural University Laboratory Animal Care and Use (Beijing, China).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 Ingredient composition and nutrient analysis of basal diets (as fed, %)

Items[a]	Diet	
	d 0-14	d 15-28
Corn	59.82	64.32
Soybean meal	15.00	15.80
Extruded soybean	6.30	6.00
Fish meal	4.00	3.50
Whey powder	4.00	3.15
Soybean protein concentrate	4.80	2.80
Soybean oil	2.20	0.90
Dicalcium phosphate	1.15	1.00
Limestone	0.82	0.60
Salt	0.30	0.30
L-lysine HCl	0.52	0.44
Methionine	0.18	0.12
Threonine	0.18	0.14
Serine	0.03	0.03
Chromic oxide	0.00	0.03
Choline chloride	0.20	0.10
Vitamin-mineral premix[b]	0.50	0.50
Total	100.00	100.00
Chemical composition[c]		
Digestible energy, MJ/Kg	3.55	3.48
SID Lysine	1.39	1.25
SID Methionine	0.49	0.41
SID Threonine	0.96	0.74
SID Serine	0.26	0.22
Crude protein	20.81	19.53
Calcium	0.84	0.70
Total phosphorous	0.65	0.61

[a]Experimental diets were corn-soybean meal based diet (CON), CON + 75 mg/kg aureomycin (ANT), CON + 1×10^8 CFU/kg *S. boulardii* mafic-1701 (SB). [b]The Vitamin-mineral premix contained (per kilogram of complete diet): vitamin A, 9000 IU; vitamin D_3, 3000 IU; vitamin E, 20.0 IU; vitamin K_3, 3.0 mg; vitamin B_1, 1.5 mg; vitamin B_2, 4.0 mg; vitamin B_6, 3.0 mg; vitamin B_{12}, 0.2 mg; niacin, 30.0 mg; pantothenic acid, 15.0 mg; folic acid, 0.75 mg; biotin, 0.1 mg; Fe ($FeSO_4 \cdot H_2O$), 75.0 mg; Cu ($CuSO_4 \cdot 5H_2O$), 150 mg; Zn ($ZnSO_4 \cdot 7H_2O$), 90 mg; Mn ($MnSO_4$), 60.0 mg; I (KI), 0.35 mg; Se (Na_2SeO_3), 0.30 mg.

[c]Except digestible energy, all nutrient levels were measured

Table 2 Effect of *S. boulardii* mafic-1701 on growth performance in weaned piglets[1]

Item	CON	ANT	SB	Pooled SEM	*P*-value			
					P^2	CON vs ANT	CON vs SB	ANT vs SB
d 0 to 14								
ADG (g)	311.6	347.4	320.1	0.01	0.38	0.19	0.75	0.31
ADFI (g)	488.6	527.7	476.9	0.02	0.44	0.35	0.77	0.22
F:G	1.85[a]	1.60[a]	1.47[b]	0.07	0.09	0.14	0.03	0.42
d 15 to 28								
ADG (g)	511.0	565.2	530.9	0.02	0.49	0.29	0.91	0.34
ADFI (g)	1096.4	1204.7	1112.5	0.03	0.39			
F:G	2.22[a]	2.03[ab]	1.97[b]	0.05	0.05	0.08	0.02	0.50
d 0 to 28								
ADG (g)	421.7	490.0	463.3	0.01	0.31	0.49	0.21	0.42
ADFI (g)	810.0	873.3	810.0	0.02	0.42	0.28	1.00	0.28
F:G	1.98[a]	1.82[b]	1.78[b]	0.02	0.03	< 0.01	< 0.01	0.23

[1]n = 6 per pen, In the same row, experimental diets were corn-soybean meal based diet (CON), CON + 75 mg/kg aureomycin (ANT), CON + 1 × 10^8 CFU/kg *S. boulardii* mafic-1701 (SB). In the same row, values with different small letter superscripts mean significant difference ($P < 0.05$). [2]Orthogonal contrast statement: [CON] vs. [ANT, SB]

Table 3 Effect of *S. boulardii* mafic-1701 on serum immune and antioxidant parameters in weaned piglets[1]

Item	CON	ANT	SB	Pooled SEM	*P*-value			
					P^2	CON vs ANT	CON vs SB	ANT vs SB
IgA (g/L)	1.00	1.38	1.30	0.09	0.16	0.08	0.14	0.70
IgG (g/L)	10.01	11.24	13.10	1.51	0.72	0.76	0.43	0.64
T-SOD (U/mL)	190.10[a]	207.44[ab]	224.59[b]	5.21	0.02	0.11	< 0.01	0.12
MDA (nmol/mL)	2.33[a]	1.74[ab]	1.59[b]	0.14	0.07	0.08	0.03	0.65
T-AOC (mM)	0.24	0.28	0.30	0.02	0.36	0.41	0.16	0.53
GSH-P_X (U/mL)	634.15	670.15	664.61	12.54	0.48	0.27	0.35	0.86

[1]Experimental diets were corn-soybean meal based diet (CON), CON + 75 mg/kg aureomycin (ANT), CON + 1 × 10^8 CFU/kg *S. boulardii* mafic-1701 (SB). Serum samples were collected from one piglet randomly selected from each replicate. In the same row,

values with different small letter superscripts mean significant difference ($P < 0.05$).

[2]Orthogonal contrast statement: [CON] vs. [ANT, SB]

Table 4 Effect of *S. boulardii* mafic-1701 on inflammatory parameters in jejunum and ileum in weaned piglets[1]

	Item	CON	ANT	SB	Pooled SEM	*P*-value			
						P[2]	CON vs ANT	CON vs SB	ANT vs SB
TNF-α (ng/L)	Jejunum	200.18[a]	112.57[b]	117.33[b]	16.60	< 0.01	< 0.01	< 0.01	0.79
	Ileum	101.59	123.24	157.42	13.38	0.31	0.49	0.15	0.34
IL-8 (ng/L)	Jejunum	133.06[a]	108.04[b]	128.97[ab]	5.36	0.07	0.03	0.69	0.08
	Ileum	286.76[a]	160.73[b]	145.11[b]	28.05	0.03	0.02	0.02	0.73
IL-6 (ng/L)	Jejunum	263.60[a]	143.04[b]	92.64[b]	28.25	< 0.01	< 0.01	< 0.01	0.08
	Ileum	111.61	111.66	132.87	8.25	0.60	1.00	0.42	0.38
IL-4 (ng/L)	Jejunum	88.74	101.35	105.16	7.74	0.14	0.45	0.06	0.18
	Ileum	87.31[a]	99.18[ab]	105.40[b]	3.64	0.06	0.09	0.03	0.34

[1]Experimental diets were corn-soybean meal based diet (CON), CON + 75 mg/kg aureomycin (ANT), CON + 1×10^8 CFU/kg *S. boulardii* mafic-1701 (SB). Intestinal samples were collected from three piglets per treatment. In the same row, values with different small letter superscripts mean significant difference ($P < 0.05$). [2]Orthogonal contrast statement: [CON] vs. [ANT, SB]

Table 5 Effect of *S. boulardii* mafic-1701 on the concentration of SCFAs (mg/kg) in weaned piglets[1]

	Item	CON	ANT	SB	Pooled SEM	*P*-value			
						P[2]	CON vs ANT	CON vs SB	ANT vs SB
Acetate	Cecum	3724.49	4052.51	3494.39	112.17	0.13	0.21	0.39	0.05
	colon	4102.98[a]	4527.22[b]	4245.64[ab]	74.78	0.05	0.02	0.39	0.09
Propionate	Cecum	2241.26	2484.82	2812.71	221.88	0.60	0.67	0.32	0.57
	colon	2686.08[a]	3252.93[b]	2861.84[a]	81.83	< 0.01	< 0.01	0.27	0.01
Formate	Cecum	36.77[a]	62.54[ab]	72.37[b]	6.43	0.06	0.06	0.04	0.53
	colon	58.27	63.13	68.00	4.20	0.67	0.66	0.38	0.66
Isobutyrate	Cecum	2.89[a]	5.10[a]	15.60[b]	2.42	0.03	0.49	0.01	0.03
	colon	42.35[ab]	44.60[a]	28.09[b]	3.26	0.05	0.76	0.05	0.03
Butyrate	Cecum	870.88	1114.59	1204.74	87.96	0.29	0.27	0.14	0.68
	colon	1499.63[a]	2105.30[b]	1833.76[a]	97.96	0.03	0.01	0.13	0.19
Isovalerate	Cecum	2.15[a]	11.67[b]	5.47[ab]	1.64	0.04	0.01	0.31	0.06
	colon	36.80	36.70	22.72	4.55	0.35	0.99	0.24	0.21
Valerate	Cecum	92.16[a]	211.31[b]	235.98[b]	24.69	0.01	0.02	0.01	0.62
	colon	250.90[a]	460.89[b]	258.83[a]	35.87	0.01	0.01	0.91	0.01

[1]Experimental diets were corn-soybean meal based diet (CON), CON + 75 mg/kg aureomycin (ANT), CON + 1×10^8 CFU/kg *S. boulardii* mafic-1701 (SB). Cecum and colon digesta samples were collected from three piglets per treatment and the concentration of SCFAs were measured. In the same row, values with different small letter superscripts mean significant difference ($P < 0.05$). [2]Orthogonal contrast statement: [CON] vs. [ANT, SB]

Figures

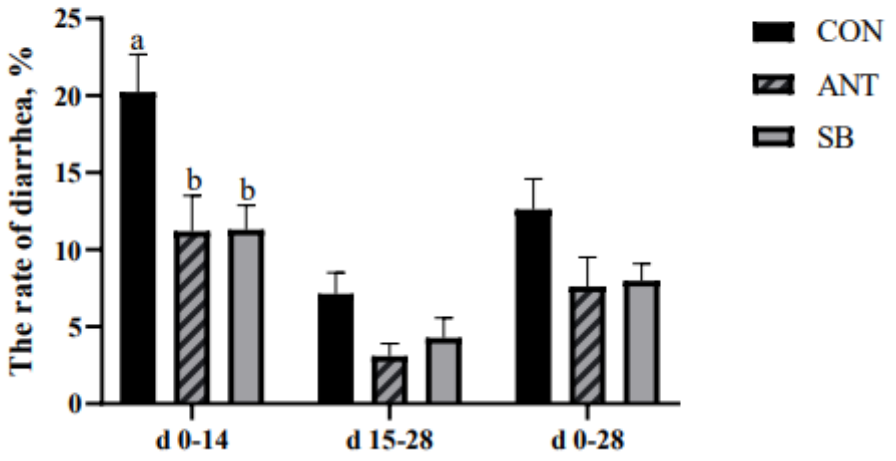

Figure 1

Effects of diet treatment on the rate of diarrhea in weaned piglets. Experimental diets were corn-soybean meal based diet (CON), CON + 75 mg/kg aureomycin (ANT), CON + 1 × 108 CFU/kg S. boulardii mafic-1701 (SB). Data are presented as the mean ± SEM, n = 6. Different superscript small letters within each group represent significantly different values (P < 0.05)

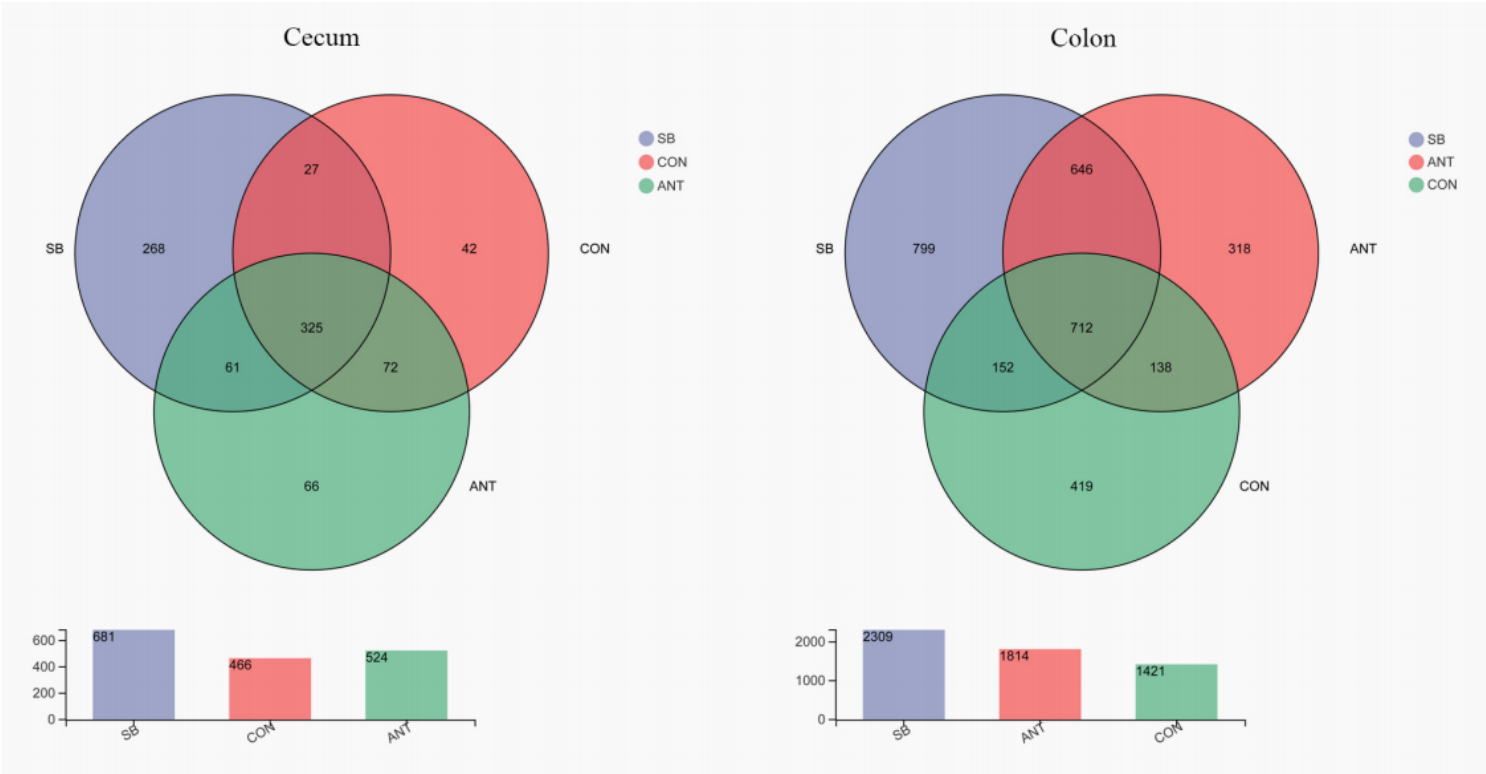

Figure 2

The bacterial core OUT community composition of the cecum and colon in weaned piglets. Venn diagrams of bacterial core OUT community among three treatment groups: corn-soybean meal based diet

(CON), CON + 75 mg/kg aureomycin (ANT), CON + 1 × 108 CFU/kg S. boulardii mafic-1701 (SB)

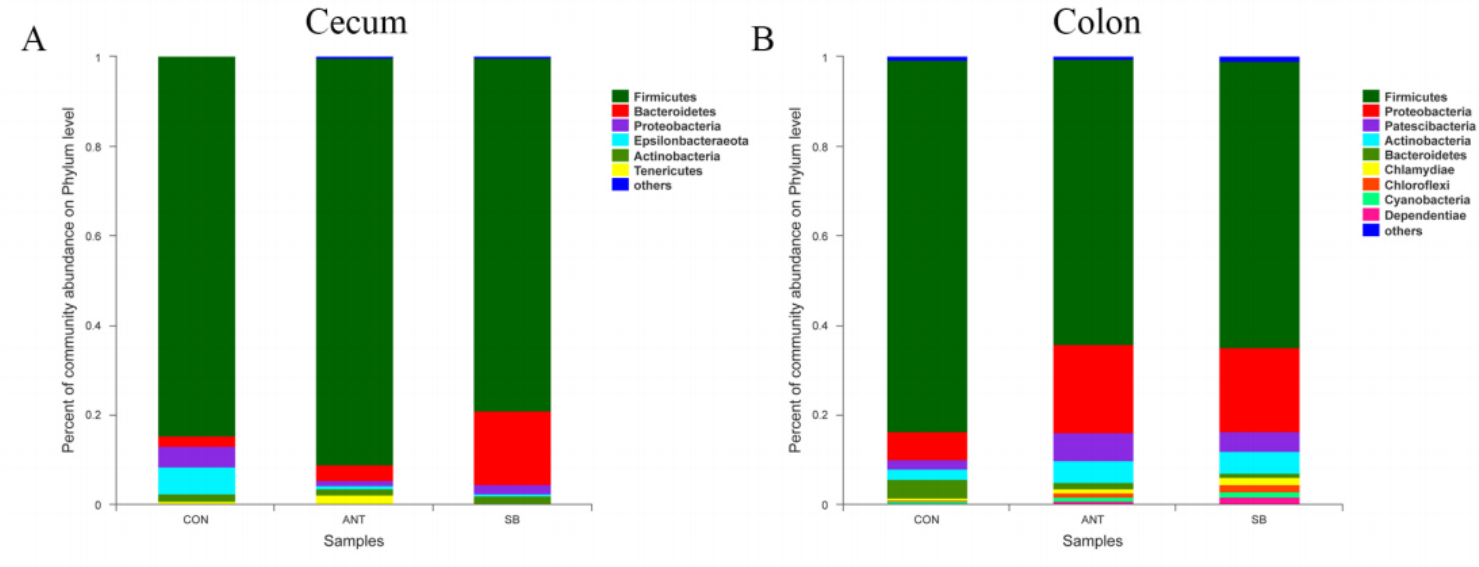

Figure 3

Characterization of communities on phylum level. Experimental diets were corn-soybean meal based diet (CON), CON + 75 mg/kg aureomycin (ANT), CON + 1 × 108 CFU/kg S. boulardii mafic-1701 (SB). Effects of diet treatment on cecum and colon bacterial community of weaned piglets at the phylum level. (A) Community barplot analysis for the cecum digesta bacterial community. (B) Community barplot analysis for the colon digesta bacterial community

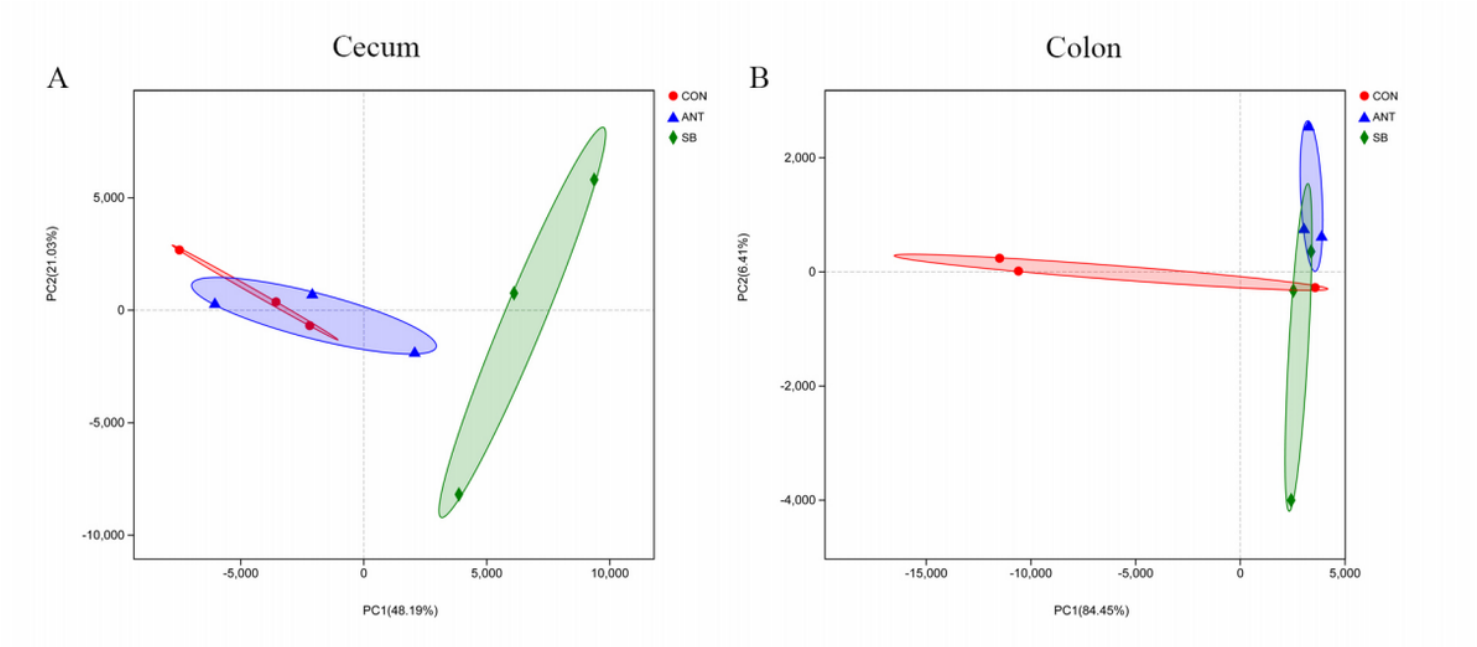

Figure 4

PCA of bacterial community. Experimental diets were corn-soybean meal based diet (CON), CON + 75 mg/kg aureomycin (ANT), CON + 1 × 108 CFU/kg S. boulardii mafic-1701 (SB), Different symbols

represent different treatment groups. (A) PCA plot for the cecum digesta bacterial community. (B) PCA plot for the colon digesta bacterial community

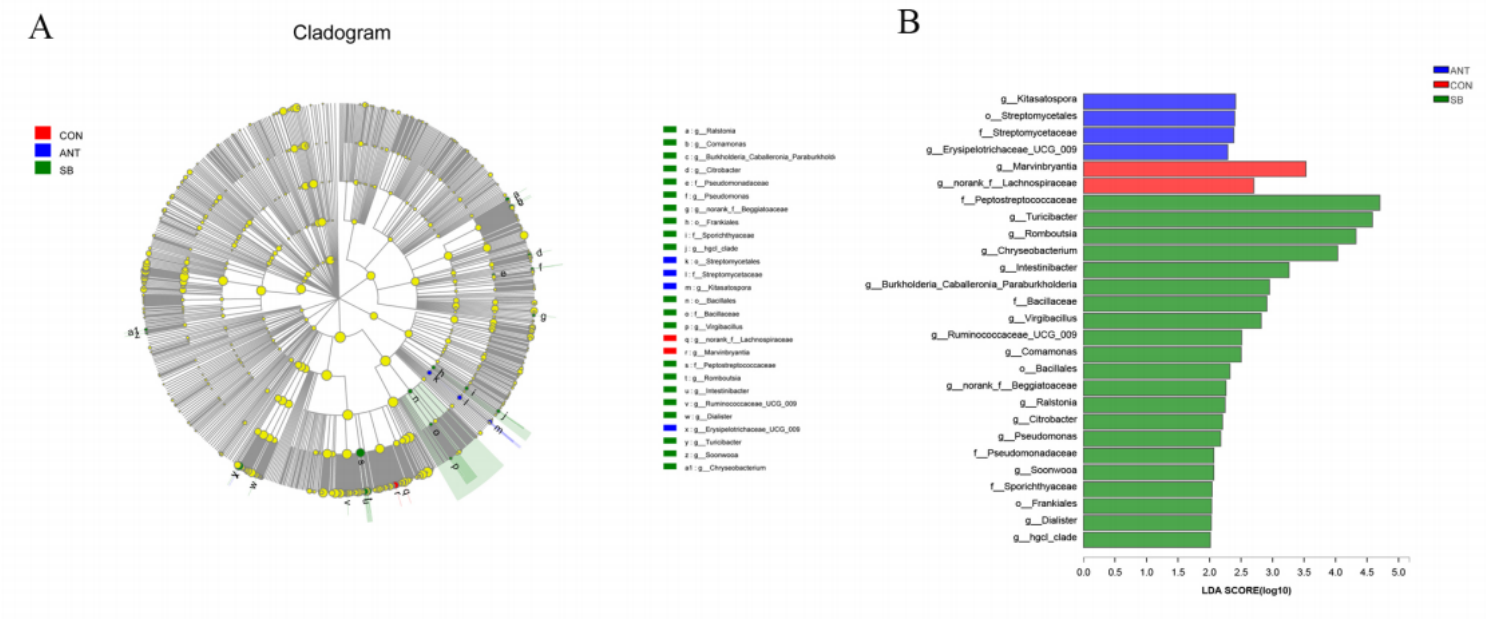

Figure 5

Different structures of cecum bacterial communities from phylum to genus level among three treatment groups. Experimental diets were corn-soybean meal based diet (CON), CON + 75 mg/kg aureomycin (ANT), CON + 1 × 108 CFU/kg S. boulardii mafic-1701 (SB). (A) Taxonomic representation of distinct bacterial with statistically significant higher abundances. (B) Histogram of LDA plots indicate scores for differentially abundant genera

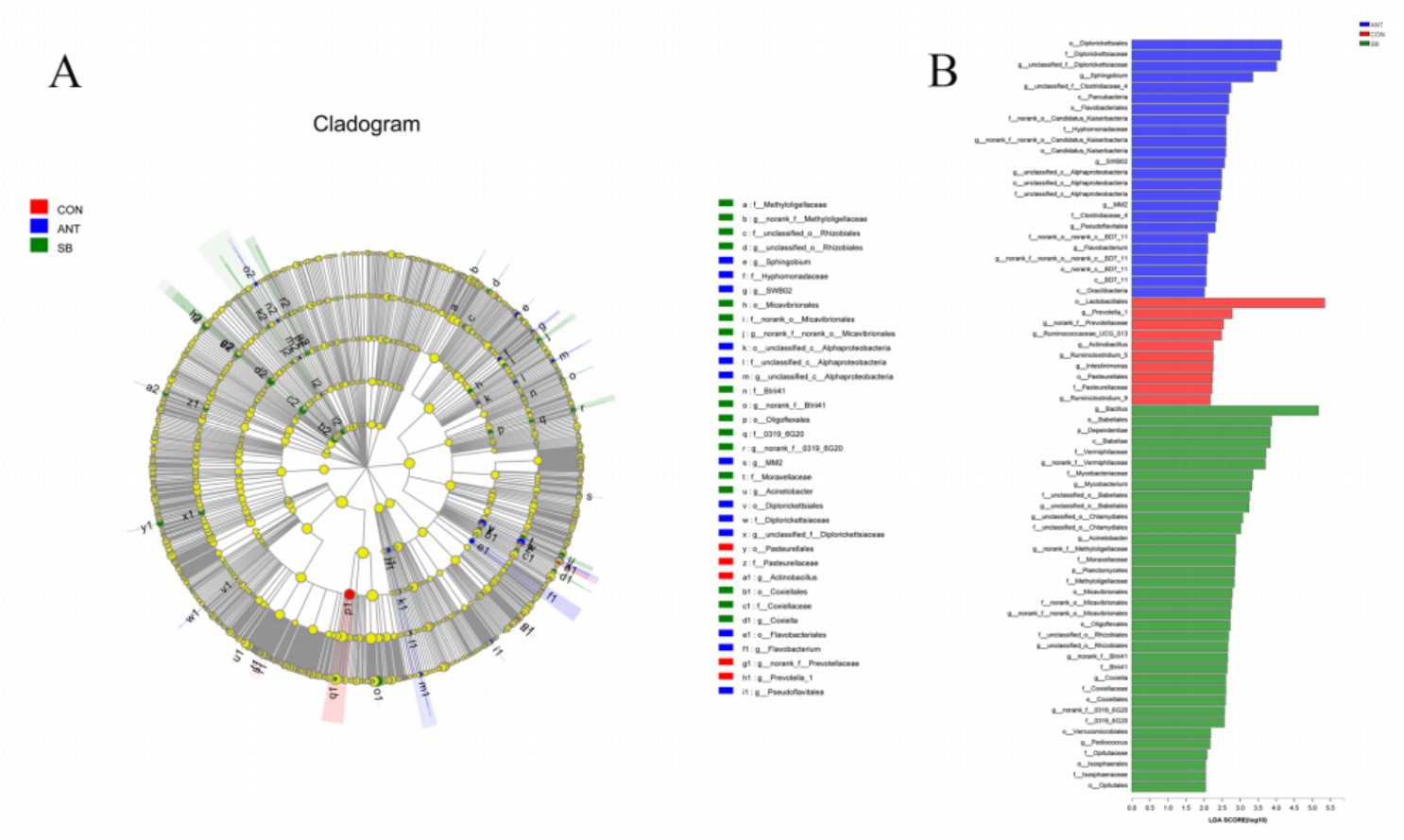

Figure 6

Different structures of colon bacterial communities from phylum to genus level among three treatment groups. Experimental diets were corn-soybean meal based diet (CON), CON + 75 mg/kg aureomycin (ANT), CON + 1 × 108 CFU/kg S. boulardii mafic-1701 (SB). (A) Taxonomic representation of distinct bacterial with statistically significant higher abundances. (B) Histogram of LDA plots indicate scores for differentially abundant genera