Administration of *Saccharomyces boulardii* mafic-1701 improves feed conversion ratio, 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 for are used as a means to improve animal health and intestinal development. Saccharomyces boulardii (S. boulardii) is a well-known probiotic, however, few studies have examined the effects of S. boulardii on weaned piglet performance. Therefore, this 28-day study compared the effects of S. boulardii mafic-1701 and aureomycin in weaned piglet diets on growth performance, antioxidant parameters, inflammation and intestinal microbiota. One hundred and eight weaned 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 × 108 CFU/kg S. boulardii mafic-1701 (SB).

Results: Compared to CON group, SB group improved feed efficiency over the entire 28 days ($P < 0.01$) and decreased the rate of diarrhea during the first two weeks ($P < 0.05$). Total superoxide dismutase concentration was markedly increased in SB group ($P < 0.05$). Moreover, compared with CON group, SB group decreased the level of pro-inflammatory cytokines interleukin-6 ($P < 0.01$) and tumor necrosis factor ($P < 0.05$) in jejunum. Supplementation with S boulardii mafic-1701 increased abundance of Bacillus and Ruminococcaceae ($P < 0.05$), whereas abundance of Clostridiaceae was decreased ($P < 0.05$). Furthermore, S boulardii mafic-1701 administration increased cecal concentration of microbial metabolites, isobutyrate and valerate ($P < 0.05$).

Conclusion: The improvement in feed conversion ratio, reduction in diarrhea rate during the first two weeks in weaned piglets provided diets supplemented with S. boulardii mafic-1701 may be associated with enhanced antioxidant activity, anti-inflammatory responses and intestinal microbial ecology.

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, with weaning age has been decreasing [1]. It is one of the most stressful matters in pig's life [1]. Some non-antibiotic solutions have been developed to maintain health newly weaned piglets, including antimicrobial peptides, prebiotics, new antibiotics, anti-virulence molecules, antibodies and probiotics [2-4]. Probiotics are defined as "friendly" live microorganisms and when administered probiotics in adequate amounts they confer a health benefit to the host before any health issue is present [5]. *Saccharomyces boulardii* (*S. boulardii*) is a safe, efficacious and non-pathogenic yeast isolated from lychee fruit in Indochina; *S. boulardii* belongs to *Saccharomyces cerevisiae* species [6]. However, *S. boulardii* exhibits several distinct physiological and metabolic characteristics and possesses a superior probiotic efficiency than *Saccharomyces cerevisiae* [6]. In particular, characteristics of *S. boulardii* that make it suitable for use in weaned piglet diets include heat tolerance and resistance to gastric acidity, bile and proteolysis [7, 8]. The degree of pH tolerance and resistance to enzyme digestion suggest it may be suited for survival in the intestinal 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 [9]. Accumulating evidence suggests oral administration *S. boulardii* may protect against antibiotic associated diarrhea and improve *Clostridium difficile*-associated colitis in animal models [10, 11]. In human studies, administration of *S. boulardii* protected against *Clostridium difficile* infection, mitigated intestinal microbiota disorder and reduced antibiotic associated diarrhea [12, 13].

The beneficial properties mentioned here would indicate *S. boulardii* is a promising probiotic- based feed additive in animal production. However, intervention studies and investigations of *S. boulardii* effects on weaned piglets remains unclear. Therefore, the objective of this study was to determine whether *S. boulardii* mafic-1701 supplementation to weaned piglet diets would improve feed conversion ratio, antioxidant capacity in serum, gut anti-inflammatory responses, microbiota composition and fermentation metabolites products in weaned piglets.

Materials And Methods

Experimental protocols of animal handling and dietary treatments 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 agar plates to screen single colonies. Colonies of *S. boulardi* mafic-1701 were inoculated in yeast extract peptone dextrose 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 mol/L NaOH. Fermentation was processed at 37 ℃ at 250 r/min with an aeration rate of 5 L/min of air. The pH was maintained at 6.5 by the addition of 3 mol/L NaOH and anti-foaming agents were automatically added when each time foam was generated. Samples were collected every 12 h to measure the biomass of *S. boulardii* mafic-1701 fermented. The yeast product used in this present study was obtained by mixing the precipitate of the fermentation broth with 21.57 kg wheat bran. The final product moisture content was controlled at 2% by heat drying.

Experimental design and diets

A randomized controlled experiment was undertaken at 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) were weaned at 28 d of age (8.5 ± 1.1 kg), and randomly assigned to one of three dietary treatment groups, based on their gender and initial body weight. Dietary treatment groups consisted of basal diet (CON), basal diet supplemented with 75 mg/kg

aureomycin (Chia Tai Group, Henan, China) (ANT) [14] and basal diet supplemented with 1×10^8 CFU/kg *S. boulardii* mafic-1701 (SB). Basal diets (Table 1) in this study were formulated to meet or exceed NRC (2012) nutritional requirements of piglets in 2 phases (d 0-14 and d 15-28) after weaning. Each treatment group consisted of 6 replicate pens and each pen consisted 3 male and 3 female piglets. All piglets were housed in identical conditions (Room temperature setpoint was 26 ℃ on the day of weaning and gradually decreased to 22 ℃ within day 7 after weaning. The humidity was held constant at 65-75%) and piglets had ad libitum to access water and feed.

Performance and diarrhea incidence

Piglets were weighed individually on days 0, 14 and 28; pen feed disappearance was also determined on days 0, 14 and 28. Average daily gain (ADG), average daily feed intake (ADFI) and feed to gain ratio (F:G) were calculated on a pen basis. To evaluate the rate of diarrhea, fecal consistency was visually assessed three times per day throughout the experiment by fixed observers blind to the treatment according to the method described by Hart and Dobb [15]. 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 [16].

Sample collection and processing

One piglet was selected from each pen with intermediate body weight and piglets were slaughtered on day 28 of the experiment. Prior to euthanasia, blood sample (7 ml) was collected via jugular venipuncture using vacutainer without anticoagulant. Blood was centrifuged at 3,000 ×g for 15 min [14]. Serum was separated and stored at -20 ℃ until analysis for serum immune parameters and antioxidant indexes. Approximately 10 g digesta from mid cecum and colon of each piglets were collected in sterile tubes, flash frozen in liquid nitrogen and stored at -80 ℃ until further analysis [17]. One aliquot of digesta samples were obtained for microbial composition analysis and additional subsamples were taken to determine the short chain fatty acids (SCFAs) in the gut. Intestinal tissue samples (3.0 cm) taken from jejunum and ileum, washed with normal saline to remove gut contents, immediately preserved in liquid nitrogen and kept at -80 ℃ for anti-inflammatory analysis.

Determination of serum immune parameters and antioxidant indexes

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

Cytokine measurement

The levels of interleukin-8 (IL-8), interleukin-4 (IL-4), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were determined in intestinal tissues. Jejunum and ileum samples were thawed and homogenized in PBS (1:9, w/v) with pH 7.4, then centrifuged at 2000 r/min for 20 min, and supernatant collected. The levels of cytokines were determined using 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 specifications. 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, raw data were quality-filtered using Trimmomatic and merged by FLASH software with the following criteria: (i) average quality score less than 20 were truncated. 50 bp sliding window was set and reads shorter than 50 bp or containing ambiguous reads were discarded; (ii) sequences longer than 10 bp were assembled based on their overlapped sequence. The maximum mismatch ratio of overlap area was 0.2. Unassembled reads were discarded; (iii) Samples were distinguished according to their barcode and primers, and reads including ambiguous bases were removed.

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 polypropylene tube and diluted 1:16 with ultrapure water (8 mL). Glass spheres were added and vortexed to homogenize the contents. Polypropylene tubes were paced in ultrasonic bath (KQ5200DE; Kunshan Ultrasonic Instrument, Jiangsu, China) at room temperature for 30 min. Then, the mixture was 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. 25 μl extracted sample solution was determined by high performance ion chromatography (ICS-3000; Dionex, USA) with a conductivity detector. Finally, the concentration of SCFAs were calculated and normalized to intestinal content weight as milligram per kilogram.

Statistical analysis

Replicate (pen) was considered the experimental unit for analysis of the difference in growth performance, the rate of diarrhea. For serum immune, antioxidant parameters, inflammatory parameters, microbial analysis and SCFAs, individual piglets were considered the experimental unit for statistical analysis. Data were analyzed by one-way ANOVA using SPASS 19.0 software (SPSS Inc., Chicago, IL, USA). Bonferroni tests were used to determine differences between CON, ANT and SB. Treatment effects were considered statistical significance if $P < 0.05$.

Results

Diarrhea incidence and growth performance

The rate of diarrhea in weaned piglets is presented in Fig. 1. During the first two weeks, SB group markedly decreased the diarrhea incidence compared to CON group ($P < 0.05$). Similarly, ANT group attenuated the diarrhea incidence ($P < 0.05$). During the last two weeks, as well as, over the entire 4 weeks, there were no significant differences in the rate of diarrhea among three treatment groups. There were no significant differences in ADFI and ADG among three treatment groups (Table 2; $P > 0.05$). During d 15 to 28 ($P < 0.05$) and d 0 to 28 ($P < 0.01$), SB group had decreased F:G compared with CON group.

Serum immune and antioxidant parameters

Dietary supplementation of aureomycin or *S. boulardii* mafic-1701 did not significantly impact serum concentration of IgA and IgG compared with CON group (Table 3). The concentration of T-SOD was increased in the serum of piglets in SB group compared to piglets in CON group ($P < 0.05$), whereas piglets in ANT group and CON group were similar ($P > 0.05$). In addition, oral aureomycin and *S. boulardii* mafic-1701 did not significantly effects the concentration of T-AOC, MDA and GSH-P_X in serum (Table 3).

Intestinal inflammatory responses

Indices related to gut inflammation were evaluated in jejunal and ileal tissue (Table 4). In this study, the levels of TNF-α ($P < 0.01$) and IL-6 ($P < 0.05$) in the jejunum were decreased significantly in ANT group compared to CON group. Similarly, SB group markedly decreased the levels of TNF-α ($P < 0.05$) and IL-6 ($P < 0.01$) in the jejunum.

Intestinal microbiota composition

The OTUs were classified for bacterial community on the basis of usable sequence at 97% similarity. The analysis of OTUs in cecum and colon digesta are shown in Fig. 2. There were 42, 66, 268 core OTUs in the cecal digesta of the CON group, ANT group, and SB group respectively and a total of 325 core OTUs were common to all treatment groups. In the colonic digesta, 712 OTUs were common among the three treatment groups with 419, 318, 799 OTUs unique to CON group, ANT group and SB group, respectively. Fig. 3 depicts the microbial composition of cecum and colon digesta samples across three treatment groups. At phylum level, *Firmicutes* was the most predominant phyla of bacterial phyla among the three

treatment groups, and *Bacteroidetes* was the second abundant phyla in ANT group and SB group. Fig. 3 also shows that *Firmicutes* and *Proteobacteria* were the dominant phyla in the colon digesta. Principal component analysis (PCA) based on Bray-Curtis distances indicated that SB group was distinctly separated in comparison to CON group and ANT group in cecum microbiota (Fig. 4). The PCA analysis of microbiota from colon digesta samples showed that CON group was relatively distinct compared to ANT group and SB group. In contrast, the colon microbiota of ANT group and SB group was more similar. Differences in the relative abundance of microbiota in cecum and colon digesta samples among three treatment groups are 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 cecal digesta samples (Fig. 5), the proportion from the *Bacillaceae* family to *Bacillales* order was significantly increased in SB group ($P < 0.05$). Moreover, genus within *Ruminococcaceae* family and genus within *Turicibacter* family were enriched in SB group ($P < 0.05$). In colon digesta samples (Fig. 6), the proportion of *Bacillus* genus was significantly increased with *S. boulardii* mafic-1701 ($P < 0.05$), while greater relative abundance of *Lactobacillales* order and *Prevotella* genus were observed in CON group ($P < 0.05$). In addition, the abundance of *Clostridiaceae* family and genus within the *Clostridiaceae* family was significantly enriched in ANT group ($P < 0.05$).

Concentrations of fermentation metabolites products

SCFAs in the cecum and colon digesta are presented in Table 5. Piglets fed *S. boulardii* mafic-1701 had higher concentration of isobutyrate and valerate in cecum digesta than piglets fed control diet ($P < 0.05$). Additionally, the concentration of propionate ($P < 0.01$) and butyrate ($P < 0.05$) in colonic digesta of aureomycin fed piglets were higher compared to CON group.

Discussion

S. boulardii is an important microorganism, which has known positive effects on humans [12, 13]. Unfortunately, data on the effect of *S. boulardii* on weaned piglets are limited. Therefore, in this study we investigated the effects of dietary *S. boulardii* mafic-1701 supplemented in the diet on weaned piglet health and gut microbiota composition over 4 weeks. The dose of *S. boulardii* mafic-1701 was selected according to the studies by Kamm et al. [18] and Hancox et al. [19] The two doses of *S. boulardii* in their studies were 1×10^7 CFU/kg and 1×10^9 CFU/kg, respectively. We selected 1×10^8 CFU/kg, the middle dose of *S. boulardii* of the two studies as the experimental treatment in this study.

In the present study, supplementation with *S. boulardii* mafic 1701 improved feed conversion ratio compared with CON group. A previous study identified that administration of yeast improved feed conversion ratio of weaned piglets [20], which corresponds with our results.

Reports on the effect of dietary supplementation with *S. boulardii* on the rate of diarrhea of weaned piglets are limited. The current study demonstrated that both SB group and ANT group significantly decreased the rate of diarrhea during the first two weeks after weaning. However, no significant difference

was observed among treatment groups during the last two weeks. A previous study demonstrated that approximately two weeks is required for weaned piglets to achieve metabolizable energy intake level equivalent to that prior to weaning [21]. Therefore, it can be speculated that in last two weeks, increased feed intake and adaption to the social and physical stressors of weaning contributed to the reduced weaning induced diarrhea.

It is generally known that weaning could lead to breakdown of intestinal barrier functions [22, 23]. When the intestinal barrier is damaged, microbial colonization increases the risk of inflammation [24]. In this study, we found that the level of pro-inflammatory cytokines TNF-α and IL-6 were decreased in SB group compared with CON group. These results indicated that *S. boulardii* mafic-1701 has beneficial effects on intestinal inflammation. Similarly, it has been previously shown that *S. boulardii* administration exerted anti-inflammatory properties in the intestinal [25-27]. A previous study showed that *S. boulardii* blocked nuclear factor kappa B activation and reduced colonic inflammation [25, 27]. Thus, we speculate that *S. boulardii* mafic-1701 altered the concentration of pro-inflammatory cytokines through modulation of the signaling pathway involved in pro-inflammatory responses such as inhibiting activation of the nuclear factor kappa B associated pathways. In addition, other previous studies have reported that mucus is composed of many immunomodulatory molecules with mucins forming the basic skeleton, which protect intestinal epithelial barrier integrity and reduce pro-inflammatory responses [4, 28]. Caballero-Franco et al. demonstrated that oral administration of probiotic increased mucin gene expression and secretion [29]. Therefore, it is speculated that *S. boulardii* mafic-1701 has a modulatory effect on inflammatory responses that correlates with regulation of mucin transcription [14].

Probiotics could activate the local mucosal protective mechanisms and exert beneficial effects on the host such as modulate anti-oxidation and immune responses [30, 31]. In our study, we observed that *S. boulardii* mafic-1701 and aureomycin supplementation had no effect on IgA and IgG levels in the serum. In terms of antioxidant analysis, we found that T-SOD was increased in SB group of the piglets, which suggests *S. boulardii* mafic-1701 plays a critical role in improving antioxidant capacity and protecting intestinal mucosa [31].

The diversity of the microorganisms in the mammalian gut is very large, it has been estimated that 500-1000 bacterial species inhabit the gut [32]. The gut microbiota has a symbiotic relationship with the host. Oral ingestion of a feed additive can regulate the delicate balance between host and microbes. From the results of phylum analysis, we found that the cecum microbial floras were dominated by *Firmicutes*, and is consistent with previous finding reported by Yu et al. [33]. Wang et al. report significant increase in abundance of *Firmicutes* and decrease in abundance of *Bacteroidetes* in the probiotics group [14]. In current study, compared to CON group, we found that SB group increased the abundance of *Proteobacteria* and decreased the abundance of *Firmicutes* and *Bacteroidetes* in the colon. Difference between the Wang et al. study and this study may be attributed to the use of different probiotic strains. Indeed, different probiotic strains could exert different physiological effects. Much work remains to be done to understand the function of different gut microbiota populations. From current study, *S. boulardii* mafic-1701 inclusion resulted in higher bacterial diversity in cecum and colon of piglets. The population

of *Ruminococcaceae* and *Turicibacter* were significantly increased in cecum of SB group compared to CON group. These bacteria are believed to be significant producers of SCFAs, which are intestinal epithelial energy components that have anti-inflammatory properties and protect intestinal epithelial cells [34-36]. In addition, *Ruminococcaceae* can utilize diverse polysaccharides [37]. Indeed, the yeast cell wall consists of mannose, chitin, 1,3-β-glucan and 1,6-β-glucan [6]. Therefore, the increased population of *Ruminococcaceae* in the cecum might be due to *S. boulardii* mafic-1701 being used as a substrate source to stimulate proliferation of *Ruminococcaceae*.

S. boulardii mafic-1701 inclusion showed some alterations with regard to microbiota communities. In the colon, *S. boulardii* mafic-1701 inclusion increased the abundance of *Bacillus* genus, which have excellent immunomodulatory and anti-inflammatory efficacy [38, 39]. In addition, a previous study reported that several *Bacillus* species, reduced pathogen colonization but the mechanisms by which this occurs is unclear [40]. Notably, the relative abundance of *Clostridiaceae* family, which are negatively linked with antibiotic associated diarrhea and colitis, was significantly increased in ANT group compared with SB group. It has been demonstrated that antibiotic treatment alters the composition of gut microbiota, manifesting the host susceptible to pathogen infection [22, 41]. *Lactobacillus* spp. are beneficial to the host due to their underlying effects on gut function and health [42, 43]. Nevertheless, the present study showed that the relative abundance of *Lactobacillus* genus was decreased in SB group compared with CON group. The reason for this result might be associated with the increased microbial diversity in SB group [42].

Microbially-produced SCFAs as crucial in regulating health of the host and play a central role in gut metabolism [44]. Previously published report indicated that probiotics can increase SCFAs production [14]. In this study, *S. boulardii* mafic-1701 supplementation increased the concentration of isobutyrate and valerate. Compared with the other two groups, ANT group increased concentrations of propionate and butyrate. The increase of SCFAs production may be associated with a lot of factors and the dietary intake is the most significant variable [45]. In additional, the principal site of microbial fermentation is proximal colon [45]. Thus, the production of SCFAs is determined by the numbers and types of microbes colonizing the colon.

Conclusion

In conclusion, diets containing *S. boulardii* mafic-1701 promoted feed conversion ratio, improved antioxidant activity and anti-inflammatory responses in weaned piglets. The increased diversity of intestinal microbiota and their fermentation products and the higher abundance of *Ruminococcaceae* spp. and *Bacillus* spp. with *S. boulardii* mafic-1701 supplementation may facilitate maturation of the digestive system of piglets in the subsequent growing phases.

Abbreviations

S. boulardii: *Saccharomyces boulardii*; ADG: Average daily gain; ADFI: Average daily feed intake; F:G: Feed to gain ratio; SCFAs: Short chain fatty acids; 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.

Declarations

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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.

References

1. Hu J, Ma L, Nie Y, Chen J, Zheng W, Wang X, et al. A microbiota-derived bacteriocin targets the host to confer diarrhea resistance in early-weaned Piglets. Cell Host Microbe. 2018;24:817-832 e8.
2. Gresse R, Chaucheyras-Durand F, Fleury MA, Van de Wiele T, Forano E, Blanquet-Diot S. Gut microbiota dysbiosis in postweaning piglets: understanding the keys to health. Trends Microbiol. 2017;25:851-873.
3. Wang T, Teng K, Liu Y, Shi W, Zhang J, Dong E, et al. *Lactobacillus plantarum* PFM 105 promotes intestinal development through modulation of gut microbiota in weaning piglets. Front Microbiol. 2019;10:90.
4. Yu H, Wang Y, Zeng X, Cai S, Wang G, Liu L, et al. Therapeutic administration of the recombinant antimicrobial peptide microcin J25 effectively enhances host defenses against gut inflammation and epithelial barrier injury induced by enterotoxigenic *Escherichia coli* infection. The FASEB Journal. 2019;34:1018-1037.
5. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11:506-514.
6. Czerucka D, Piche T, Rampal P. Review article: yeast as probiotics – *Saccharomyces boulardii*. Aliment Pharmacol Ther. 2007;26:767-778.
7. Lazo-Velez MA, Serna-Saldivar SO, Rosales-Medina MF, Tinoco-Alvear M, Briones-Garcia M. Application of *Saccharomyces cerevisiae var. boulardii* in food processing: a review. J Appl Microbiol. 2018;125:943-951.
8. Fratianni F, Cardinale F, Russo I, Iuliano C, Tremonte P, Coppola R, et al. Ability of synbiotic encapsulated *Saccharomyces cerevisiae boulardii* to grow in berry juice and to survive under simulated gastrointestinal conditions. J Microencapsul. 2014;31:299-305.
9. Offei B, Vandecruys P, De Graeve S, Foulquie-Moreno MR, Thevelein JM. Unique genetic basis of the distinct antibiotic potency of high acetic acid production in the probiotic yeast *Saccharomyces cerevisiae var. boulardii*. Genome Res. 2019;29:1478-1494.
10. Castagliuolo I, Riegler MF, Valenick L, LaMont JT, Pothoulakis C. *Saccharomyces boulardii* protease inhibits *Clostridium difficile* toxin A effects in the rat ileum. Infect Immun. 1996;64:5225-5232.
11. Koon HW, Su B, Xu C, Mussatto CC, Tran DH, Lee EC, et al. Probiotic *Saccharomyces boulardii* CNCM I-745 prevents outbreak-associated *Clostridium difficile*-associated cecal inflammation in hamsters. Am J Physiol Gastrointest Liver Physiol. 2016;311:G610-G623.
12. Kabbani TA, Pallav K, Dowd SE, Villafuerte-Galvez J, Vanga RR, Castillo NE, et al. Prospective randomized controlled study on the effects of *Saccharomyces boulardii* CNCM I-745 and amoxicillin-clavulanate or the combination on the gut microbiota of healthy volunteers. Gut Microbes. 2017;8:17-32.

13. McFarland LV. Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of *Clostridium difficile* disease. Am J Gastroenterol. 2006;101:812-822.
14. Wang S, Yao B, Gao H, Zang J, Tao S, Zhang S, et al. Combined supplementation of *Lactobacillus fermentum* and *Pediococcus acidilactici* promoted growth performance, alleviated inflammation, and modulated intestinal microbiota in weaned pigs. BMC Vet Res. 2019;15:239.
15. Hart GK, Dobb GJ. Effect of a fecal bulking agent on diarrhea during enteral feeding in the critically ill. Jpen-Parenter Enter. 1988;12:465.
16. Pan L, Zhao PF, Ma XK, Shang QH, Xu YT, Long SF, et al. Probiotic supplementation protects weaned pigs against enterotoxigenic *Escherichia coli* K88 challenge and improves performance similar to antibiotics. J Anim Sci. 2017;95:2627-2639.
17. Wang J, Liu Y, Yang Y, Bao C, Cao Y. High-level expression of an acidic thermostable xylanase in *Pichia pastoris* and its application in weaned piglets. J Anim Sci. 2020;98.
18. Kamm K, Hoppe S, Breves G, Schroder B, Schemann M. Effects of the probiotic yeast *Saccharomyces boulardii* on the neurochemistry of myenteric neurones in pig jejunum. Neuroga Stroent Motil. 2004;16:53-60.
19. Hancox LR, Le Bon M, Richards PJ, Guillou D, Dodd CE, Mellits KH. Effect of a single dose of *Saccharomyces cerevisiae* var. *boulardii* on the occurrence of porcine neonatal diarrhoea. Animal. 2015;9:1756-1759.
20. Jiang Z, Wei S, Wang Z, Zhu C, Hu S, Zheng C, et al. Effects of different forms of yeast *Saccharomyces cerevisiae* on growth performance, intestinal development, and systemic immunity in early-weaned piglets. J Anim Sci Biotechnol. 2015;6:47.
21. Campbell JM, Crenshaw JD, Polo J. The biological stress of early weaned piglets. J Anim Sci Biotechnol. 2013;4:19.
22. Wang J, Ji H, Wang S, Liu H, Zhang W, Zhang D, et al. Probiotic *Lactobacillus plantarum* promotes intestinal barrier function by strengthening the epithelium and modulating gut microbiota. Front Microbiol. 2018;9:1953.
23. Spreeuwenberg MA, Verdonk JM, Gaskins HR, Verstegen MWA. Small intestine epithelial barrier function is compromised in pigs with low feed intake at weaning. J Nutr. 2001;131:1520.
24. Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nat Rev Immunol. 2014;14:141-153.
25. Sougioultzis S, Simeonidis S, Bhaskar KR, Chen X, Anton PM, Keates S, et al. *Saccharomyces boulardii* produces a soluble anti-inflammatory factor that inhibits NF-kappaB-mediated IL-8 gene expression. Biochem Biophys Res Commun. 2006;343:69-76.
26. Rodriguez-Nogales A, Algieri F, Garrido-Mesa J, Vezza T, Utrilla MP, Chueca N, et al. Intestinal anti-inflammatory effect of the probiotic *Saccharomyces boulardii* in DSS-induced colitis in mice: impact on microRNAs expression and gut microbiota composition. J Nutr Biochem. 2018;61:129-139.
27. Lee SK, Kim YW, Chi SG, Joo YS, Kim HJ. The effect of *Saccharomyces boulardii* on human colon cells and inflammation in rats with trinitrobenzene sulfonic acid-induced colitis. Dig Dis Sci.

2009;54:255-263.

28. Johansson ME, Hansson GC. Immunological aspects of intestinal mucus and mucins. Nat Rev Immunol. 2016;16:639-649.
29. Caballero-Franco C, Keller K, De Simone C, Chadee K. The VSL#3 probiotic formula induces mucin gene expression and secretion in colonic epithelial cells. Am J Physiol Gastrointest Liver Physiol. 2007;292:G315-22.
30. Gobi N, Vaseeharan B, Chen JC, Rekha R, Vijayakumar S, Anjugam M, et al. Dietary supplementation of probiotic *Bacillus licheniformis* Dahb1 improves growth performance, mucus and serum immune parameters, antioxidant enzyme activity as well as resistance against *Aeromonas hydrophila* in tilapia *Oreochromis mossambicus*. Fish Shellfish Immunol. 2018;74:501-508.
31. Rajput IR, Li YL, Xu X, Huang Y, Zhi WC, Yu DY, et al. Supplementary effects of *Saccharomyces boulardii* and *Bacillus subtilis* B10 on digestive enzyme activities, antioxidation capacity and blood homeostasis in broiler. Int J Agric Biol. 2013;15:231-237.
32. Kim HB, Isaacson RE. The pig gut microbial diversity: understanding the pig gut microbial ecology through the next generation high throughput sequencing. Vet Microbiol. 2015;177:242-251.
33. Yu L, Zhao XK, Cheng ML, Yang GZ, Wang B, Liu HJ, et al. *Saccharomyces boulardii* administration changes gut microbiota and attenuates D-galactosamine-induced liver injury. Sci Rep. 2017;7:1359.
34. Suchodolski JS, Markel ME, Garcia-Mazcorro JF, Unterer S, Heilmann RM, Dowd SE, et al. The fecal microbiome in dogs with acute diarrhea and idiopathic inflammatory bowel disease. PLoS One. 2012;7:e51907.
35. Lo Presti A, Zorzi F, Del Chierico F, Altomare A, Cocca S, Avola A, et al. Fecal and mucosal microbiota profiling in irritable bowel syndrome and inflammatory bowel disease. Front Microbiol. 2019;10:1655.
36. Rossi G, Pengo G, Caldin M, Palumbo Piccionello A, Steiner JM, Cohen ND, et al. Comparison of microbiological, histological, and immunomodulatory parameters in response to treatment with either combination therapy with prednisone and metronidazole or probiotic VSL#3 strains in dogs with idiopathic inflammatory bowel disease. PLoS One. 2014;9:e94699.
37. Shang Q, Shan X, Cai C, Hao J, Li G, Yu G. Dietary fucoidan modulates the gut microbiota in mice by increasing the abundance of *Lactobacillus* and *Ruminococcaceae*. Food Funct. 2016;7:3224-3232.
38. Shinde T, Perera AP, Vemuri R, Gondalia SV, Beale DJ, Karpe AV, et al. Synbiotic supplementation with prebiotic green banana resistant starch and probiotic *Bacillus coagulans* spores ameliorates gut inflammation in mouse model of inflammatory bowel diseases. Eur J Nutr. 2020.
39. Rhayat L, Maresca M, Nicoletti C, Perrier J, Brinch KS, Christian S, et al. Effect of *Bacillus subtilis* strains on intestinal barrier function and inflammatory response. Front Immunol. 2019;10:564.
40. Piewngam P, Zheng Y, Nguyen TH, Dickey SW, Joo HS, Villaruz AE, et al. Pathogen elimination by probiotic *Bacillus* via signalling interference. Nature. 2018;562:532-537.
41. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol. 2009;9:313.

42. Liu P, Zhao J, Wang W, Guo P, Lu W, Wang C, et al. Dietary corn bran altered the diversity of microbial communities and cytokine production in weaned Pigs. Front Microbiol. 2018;9:2090.
43. Su Y, Yao W, Perez-Gutierrez ON, Smidt H, Zhu WY. Changes in abundance of *Lactobacillus* spp. and *Streptococcus suis* in the stomach, jejunum and ileum of piglets after weaning. FEMS Microbiol Ecol. 2008;66:546-555.
44. Cho I, Yamanishi S, Cox L, Methe BA, Zavadil J, Li K, et al. Antibiotics in early life alter the murine colonic microbiome and adiposity. Nature. 2012;488.
45. Wong JMW, De Souza R, Kendall CWC, Emam A, Jenkins DJA. Colonic health: fermentation and short chain fatty acids. J Clin Gastroenterol. 2006;40:235-243.

Tables

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

	Diet	
Items[a]	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 control 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 body weight, kg	8.4	8.6	8.5	0.26	0.97	0.82	0.89	0.93
d 14 body weight, kg	12.8	13.6	13.0	0.38	0.71	1.00	1.00	1.00
d 28 body weight, kg	20.1	22.1	21.4	0.57	0.37	0.51	1.00	1.00
d 0 to 14								
ADG, g/d	311.6	347.4	320.1	0.01	0.38	0.56	1.00	0.92
ADFI, g/d	488.6	527.7	476.9	0.02	0.44	1.00	1.00	0.67
F:G	1.58	1.52	1.47	0.04	0.56	1.00	0.88	1.00
d 15 to 28								
ADG, g/d	511.0	565.2	579.1	0.02	0.28	0.69	0.40	1.00
ADFI, g/d	1096.4	1204.7	1112.5	0.03	0.39	0.63	1.00	0.85
F:G	2.17^{a}	2.14^{ab}	1.92^{b}	0.04	0.02	1.00	0.04	0.07
d 0 to 28								
ADG, g/d	421.7	490.0	463.3	0.01	0.31	0.15	0.63	1.00
ADFI, g/d	810.0	873.3	810.0	0.02	0.42	0.83	1.00	0.83
F:G	1.92^{a}	1.82^{b}	1.78^{b}	0.02	< 0.01	0.02	< 0.01	0.76

[1]n = 6 per pen, In the same row, experimental diets were control 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	0.24	0.41	1.00
IgG, g/L	10.01	11.24	13.10	1.51	0.72	1.00	1.00	1.00
T-SOD, U/mL	190.10^{a}	207.44^{ab}	224.59^{b}	5.21	0.02	0.33	0.01	0.35
MDA, nmol/mL	2.33	1.74	1.59	0.14	0.07	0.23	0.10	1.00
T-AOC, mM	0.24	0.28	0.30	0.02	0.36	1.00	0.49	1.00
GSH-P_X, U/mL	634.15	670.15	664.61	12.54	0.48	0.81	1.00	1.00

[1]Experimental diets were control 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 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	1.00
	Ileum	101.59	123.24	157.42	13.38	0.31	1.00	0.44	1.00
IL-8, ng/L	Jejunum	133.06	108.04	128.97	5.36	0.07	0.10	1.00	0.25
	Ileum	286.76	160.73	145.11	28.05	0.03	0.06	0.06	1.00
IL-6, ng/L	Jejunum	263.60^{a}	143.04^{b}	92.64^{b}	28.25	< 0.01	0.01	< 0.01	0.23
	Ileum	111.61	111.66	132.87	8.25	0.60	1.00	1.00	1.00
IL-4, ng/L	Jejunum	88.74	101.35	125.38	7.74	0.14	1.00	0.18	0.54
	Ileum	87.31	99.18	105.40	3.64	0.06	0.26	0.08	1.00

[1]Experimental diets were control 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	0.61	1.00	0.15
	colon	4102.98	4527.22	4245.64	74.78	0.05	0.06	1.00	0.27
Propionate	Cecum	2241.26	2484.82	2812.71	221.88	0.60	1.00	0.97	1.00
	colon	2686.08^a	3252.93^b	2861.84^a	81.83	< 0.01	< 0.01	0.82	0.04
Formate	Cecum	36.77	62.54	72.37	6.43	0.06	0.17	0.12	1.00
	colon	58.27	63.13	68.00	4.20	0.67	1.00	1.00	1.00
Isobutyrate	Cecum	2.89^a	5.10^{ab}	15.60^b	2.42	0.03	1.00	0.04	0.09
	colon	42.35	44.60	28.09	3.26	0.05	1.00	0.15	0.08
Butyrate	Cecum	870.88	1114.59	1204.74	87.96	0.29	0.80	0.41	1.00
	colon	1499.63^a	2105.30^b	1833.76^{ab}	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	0.04	0.92	0.18
	colon	36.80	36.70	22.72	4.55	0.35	1.00	0.70	0.63
Valerate	Cecum	92.16^a	211.31^b	235.98^b	24.69	0.01	0.04	0.03	1.00
	colon	250.90^a	460.89^b	258.83^a	35.87	0.01	0.03	1.00	0.02

[1]Experimental diets were control 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

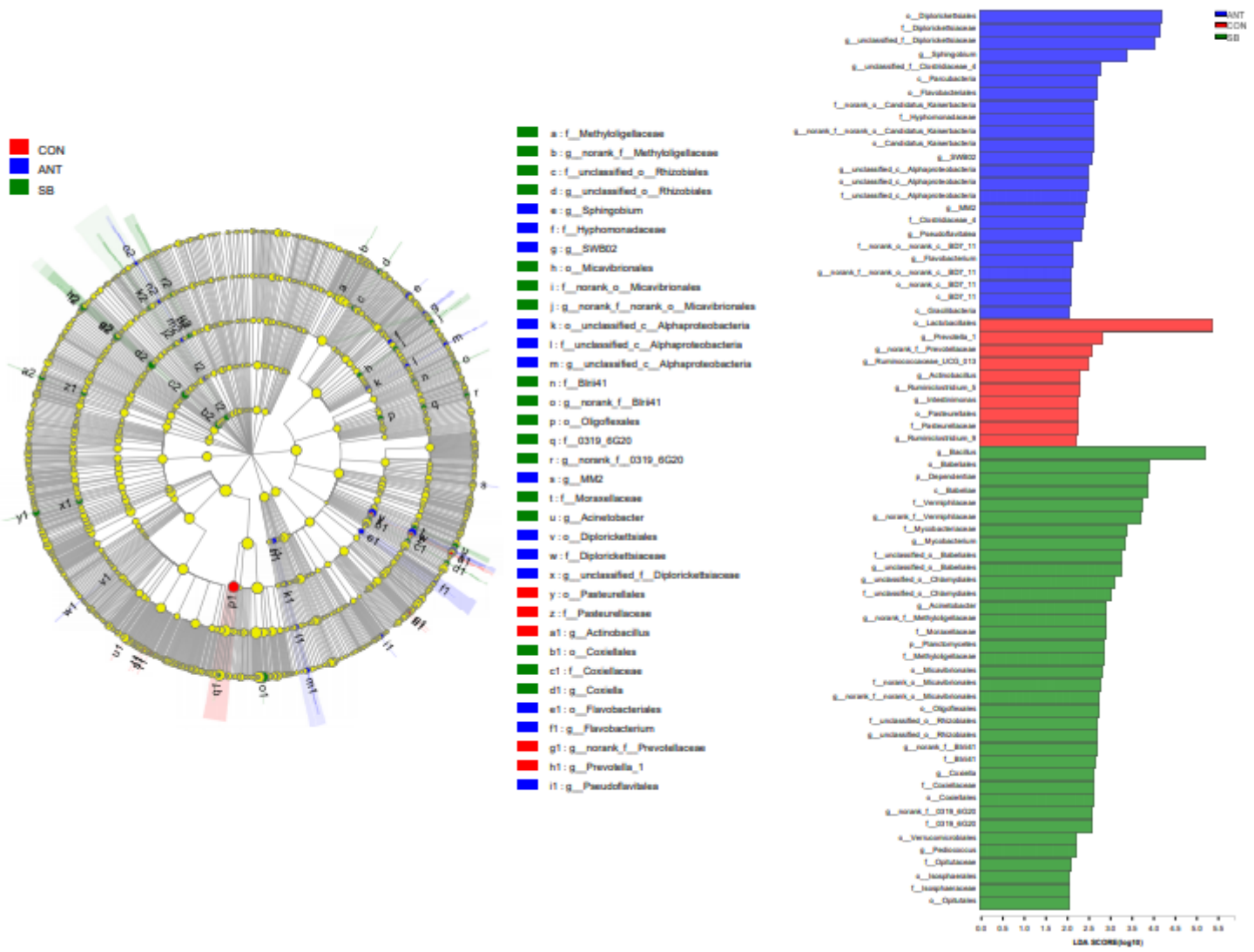

Figure 1

Different structures of colon bacterial communities from phylum to genus level among three treatment groups. Experimental diets were control 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

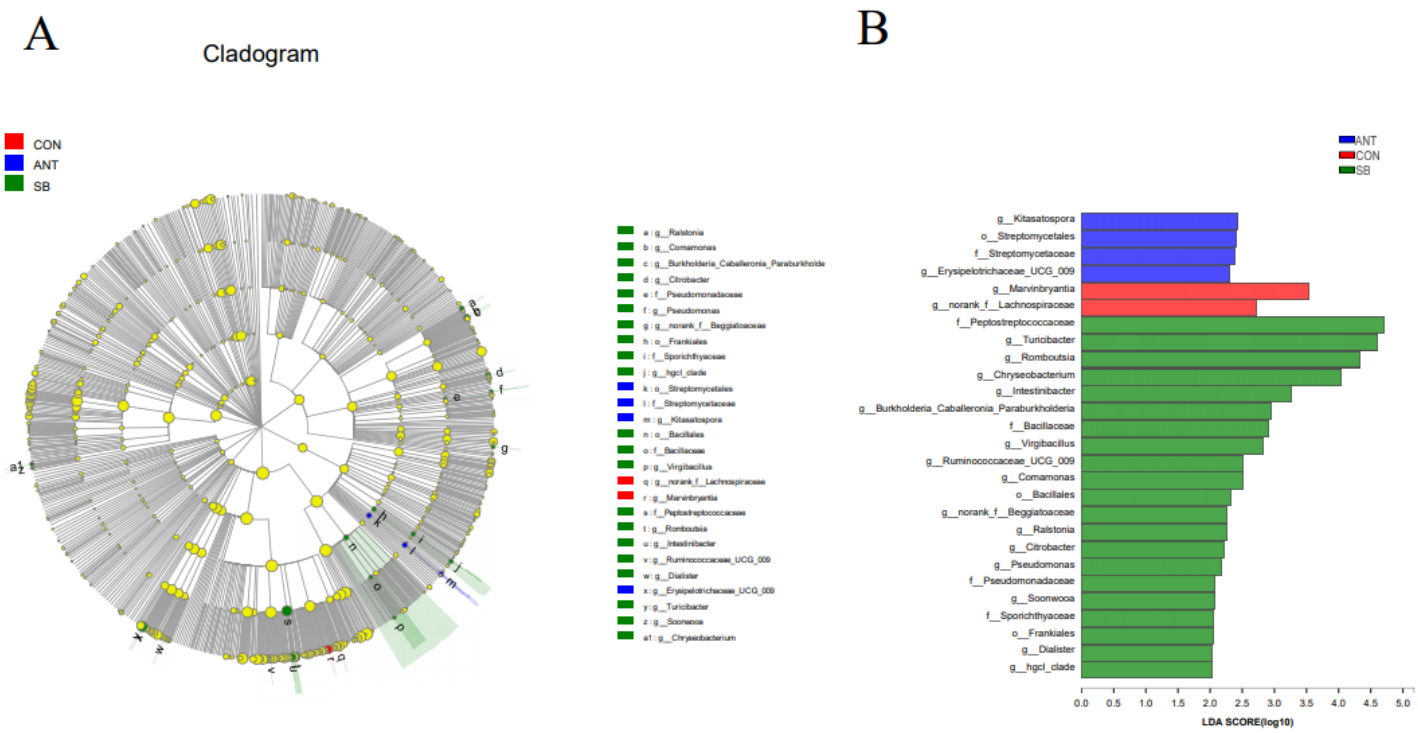

Figure 2

Different structures of cecum bacterial communities from phylum to genus level among three treatment groups. Experimental diets were control 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

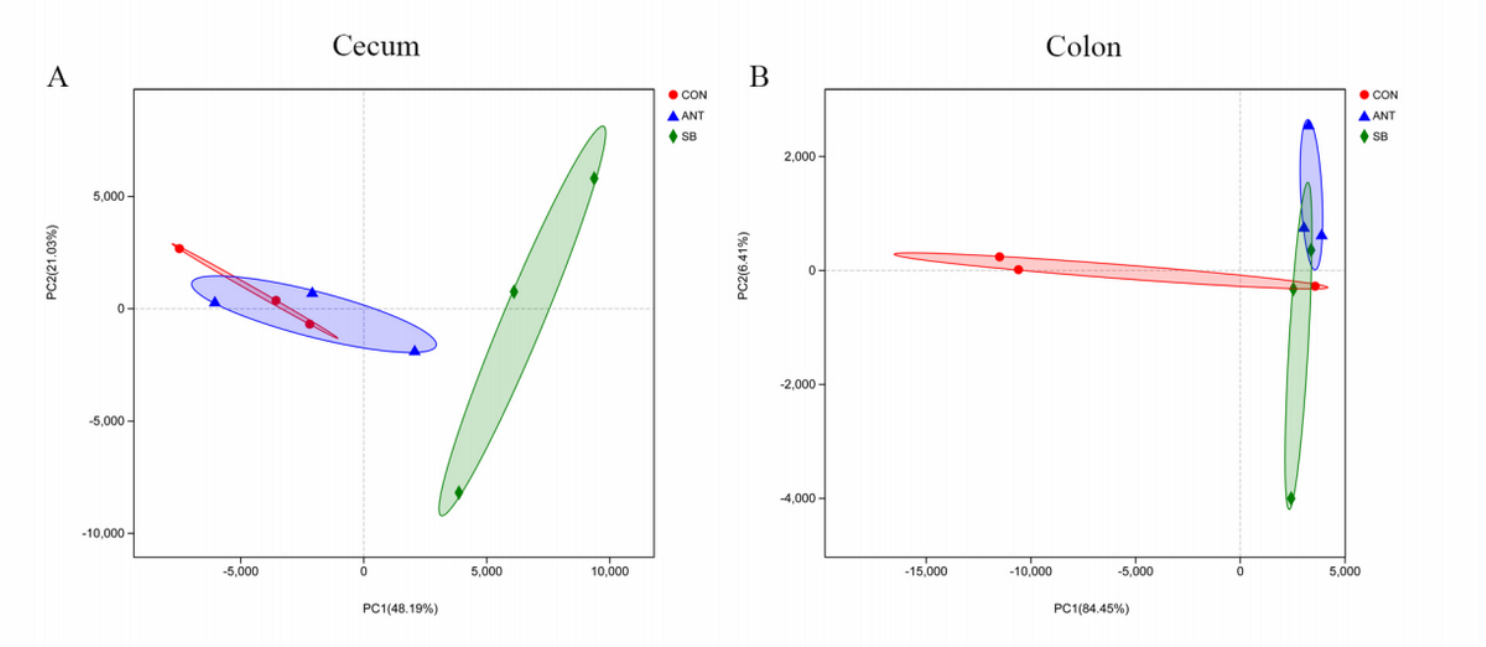

Figure 3

PCA of bacterial community. Experimental diets were control 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

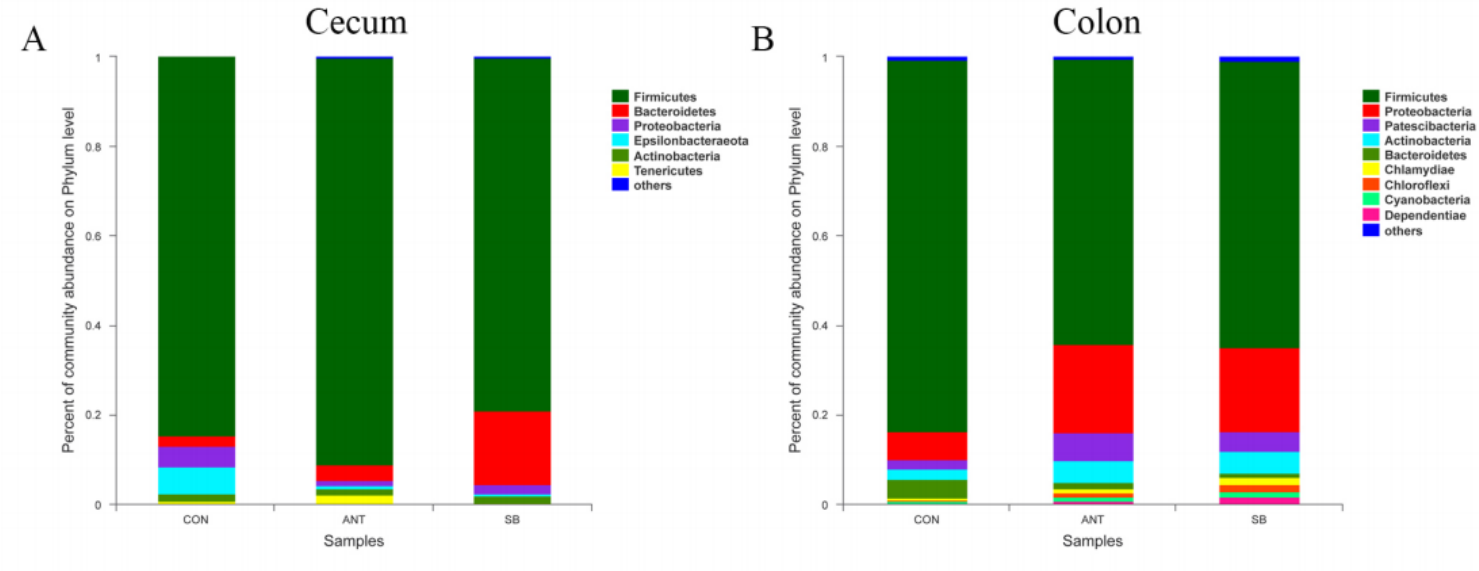

Figure 4

Characterization of communities on phylum level. Experimental diets were control 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

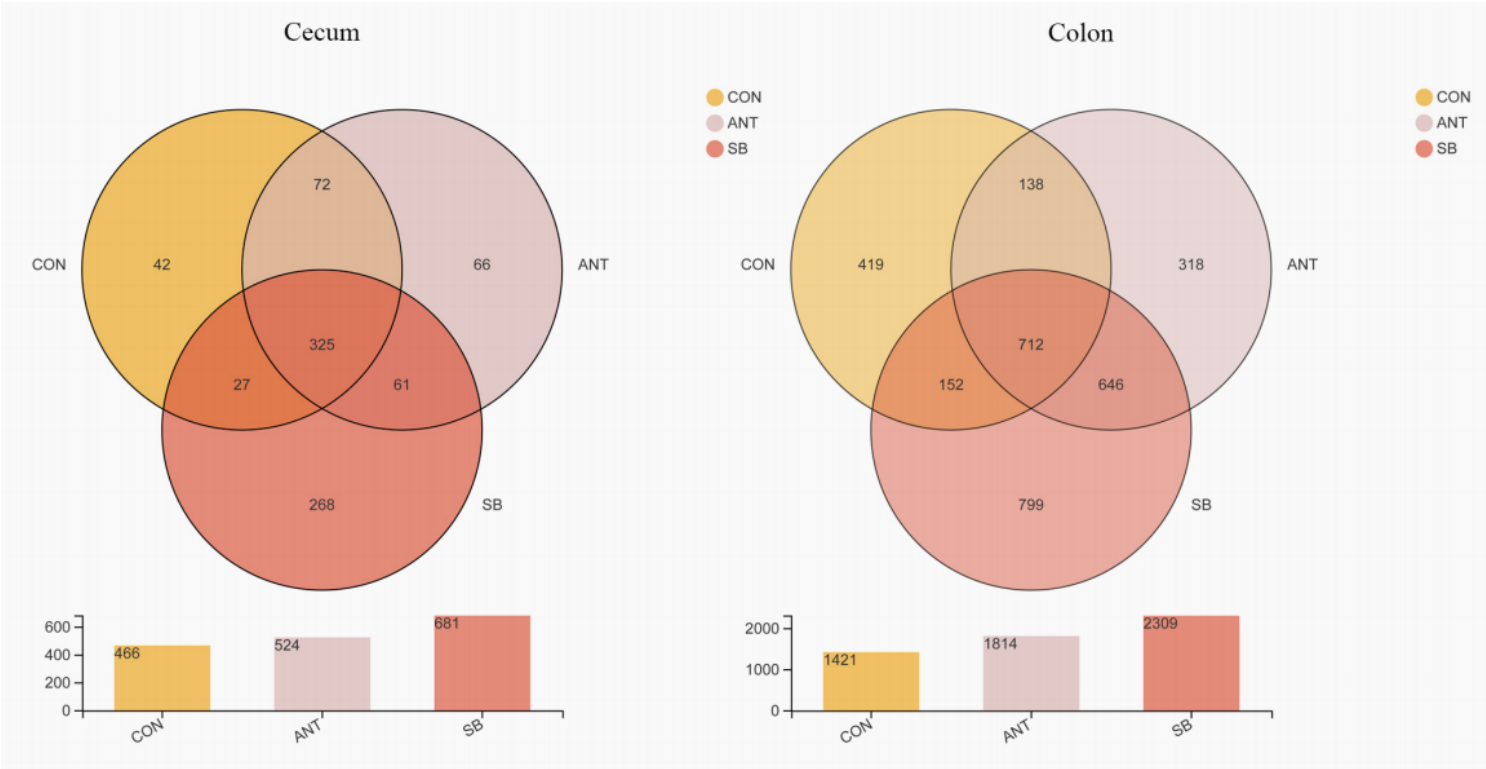

Figure 5

The bacterial core OTU community composition of the cecum and colon in weaned piglets. Venn diagrams of bacterial core OTU community among three treatment groups: control diet (CON), CON + 75 mg/kg aureomycin (ANT), CON + 1 × 108 CFU/kg S. boulardii mafic-1701 (SB)

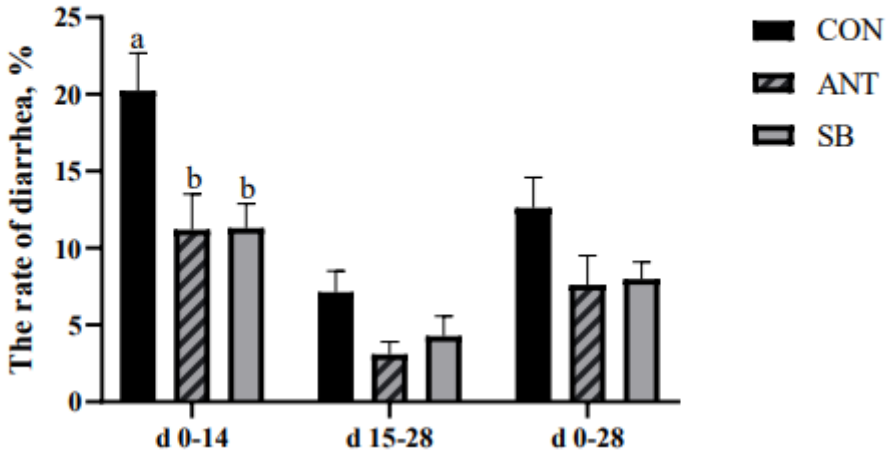

Figure 6

Effects of diet treatment on the rate of diarrhea in weaned piglets. Experimental diets were control 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 ($P < 0.05$)