Effects of combined application of benzoic acid and 1-monolaurin on growth performance, nutrient digestibility, gut microflora and systemic inflammation levels of weaned piglets

Kai Wei
Huazhong Agricultural University

Xia Yang
Huazhong Agricultural University

Huasheng Zhao
ABNA Feed (Shanghai) Co., Ltd. Zhumadian Mill

Huanchun Chen
Huazhong Agricultural University

Weicheng Bei (beiwc@mail.hzau.edu.cn)
Huazhong Agricultural University

Research Article

Keywords: Benzoic acid, growth performance, nutrient digestibility, weaned piglets, 1-monolaurin

Posted Date: July 3rd, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3118092/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Background

Our previous study found that benzoic acid and 1-monolaurin have synergistic bactericidal effect, and the improvement effect of benzoic acid and 1-monolaurin on the growth performance and diarrhea of weaned piglets was better than that of the two feeding alone. However, it is not clear how the combination of benzoic acid and 1-monolaurin affects the growth performance of weaned piglets. Therefore, 100 weaned piglets (mean weight 7.03 ± 1.04 kg, mean weaning age 26 d) were randomly divided into 2 groups: (1) basal diet control group (CON); (2) basal diet supplemented with 0.6% benzoic acid and 0.1% 1-monolaurin experimental group (CA). The experiment lasted from day 1 to day 28 after weaning. The effects of benzoic acid and 1-monolaurin supplementation on growth performance, apparent digestibility of nutrients, intestinal flora composition and function, and systemic inflammation level of weaned piglets were investigated.

Results

And it turns out, the feed conversion efficiency of piglets in CA group during 15–28 d and 1–28 d after weaning was significantly higher than that in CON group (P < 0.05), and the proportion and frequency of diarrhea of piglets in CA group 1–14 days after weaning were significantly reduced (P < 0.05). The apparent digestibility of dry matter, organic matter and crude protein of piglets in CA group were significantly higher than that in CON group on the 14 d and 28 d of the experiment (P < 0.05). The microbial composition in cecal digesta of piglets was detected. The results showed that piglets in CA group were significantly enriched in \textit{g}_{YRC22} at day 14 and \textit{g}_{Treponema}, \textit{g}_{Pseudomonas} and \textit{g}_{Lachnobacterium} at day 28 (P < 0.05; log LDA > 2). There was no significant difference in the content of short-chain fatty acids between CON and CA group. In addition, compared with CON group, serum IL-1β level in CA group was significantly decreased at day 28 and serum endotoxin content was significantly decreased at day 14.

Conclusion

In conclusion, dietary supplementation of 0.6% benzoic acid and 0.1% 1-monolauryl improved growth performance and nutrient digestibility, affect gut microflora composition, and reduce systemic inflammatory response and intestinal permeability of weaned piglets. This provides a theoretical basis for the application of benzoic acid and 1-monolauryl on weaned piglets.

Background

Compared with weaning under natural conditions, stress factors such as mother-child separation, barn environment change, diet change and other stress factors jointly affect the intestinal health of piglets,
and lead to diarrhea and growth rate reduction of piglets after weaning [1–3]. Organic acids and monoglycerides have become an important class of potential feed additives in pig production due to their key molecular characteristics and multifunctional functions, including their inhibitory activities against viral and bacterial pathogens. Among them, benzoic acid is the simplest aromatic carboxylic acid with broad-spectrum antimicrobial properties [4]. It has been reported that dietary supplementation of 0.5% benzoic acid could increase the number of effective microorganisms (e.g., *lactobacillus, bifidobacterium*) and/or reduce the number of harmful microorganisms (e.g., *E. coli*) in the intestinal tract of weaned piglets [5, 6]. However, experimental results have also shown that dietary supplementation with 1% benzoic acid can improve average dairy feed intake (ADFI), body weight (BW), average dairy gain (ADG) and feed conversion efficiency (gain/feed, G/F) of piglets, but reduce the number of total aerobic bacteria, total anaerobic bacteria, lactic acid-producing bacteria and gram-negative bacteria in chylme and the concentration of acetic acid in duodenum [7]. These results indicate that proper supplementation of benzoic acid in pig diet regulated intestinal flora and affected growth performance of piglets. In addition, glycerol monolaurate effectively prevent or delay the production of exotoxins by pathogenic gram-positive bacteria, and inhibited the synthesis of staphylococcal toxins and other exoproteins at the transcriptional level [8]. The lipophilic and hydrophilic properties of 1-monolaurin make it have good penetration to bacteria, which helps benzoic acid to enter into bacteria and exert antibacterial function. The team’s previous study found that benzoic acid and 1-monolaurin had obvious synergistic antibacterial effects on *Escherichia coli* and *Staphylococcus aureus*, and the concentration of benzoic acid and 1-monolaurin combined was lower than that of the two used alone. It has been studied that benzoic acid combined with probiotics or plant essential oils can improve diarrhea and growth performance of weaned piglets better than benzoic acid alone [9–11], however, there are few reports on the effect of combined benzoic acid and glycerol monolaurate.

Based on the results of previous experiments, the purpose of this study was to explore the effects of the combined use of benzoic acid and 1-monolaurin as feed additives on growth performance, nutrient digestibility, intestinal flora and organism inflammation of piglets, with a view to clarifying the combined application value of benzoic acid and monoglycerin in weaning diets of piglets.

### Materials and methods

#### Animals, housing and diet

The study was conducted from June 2022 to August 2022 at a large-scale farm in Hubei Province, China. A total of 100 weaned piglets (mean weight 7.03 ± 1.04 kg, mean weaning age 26 d) were randomly divided into two treatment groups on the weaning day with 5 replicates in each group and 10 piglets in each replicate. The dietary formulations of the piglets in the two treatment groups were shown in Table 1, which were used to feed experimental piglets 1 to 14 days after weaning and 15 to 28 days after weaning. The control group diet was the basal diet (control group, CON), and the experimental group diet was supplemented with 0.6% benzoic acid and 0.1% 1-monolaurin on the basis of the basal diet (Complex acid group, CA). The trial lasted for 28 days. During the experiment, the piglets drink freely and
piglets were kept in a semi-enclosed nursery with cement floor, good ventilation and warmth measures, and other feeding management and immunization procedures were uniformly implemented in accordance with the regulations of the farm.

**Sample collection**

**Serum sample**

On the mornings of day 1, 15, and 29, 5 median weight piglets were randomly selected from each treatment for labeling and blood collection. 20 ml of blood was collected from the anterior vena cava in a non-heparinized vacuum tube, centrifuged at 3000 × g at 4°C for 10 min, and serum was extracted. The serum samples were immediately stored at −20°C for further analysis.

**Sample of cecal contents**

On the morning of day 1, 15 and 29, the piglets were euthanized by intravenous injection of chlorpromazine hydrochloride with 2 mg/kg piglet body weight (Shanghai Harvest Pharmaceutical Co., LTD., Shanghai, China) about 30 minutes after feeding. According to a previously published method [12], the whole intestine was removed, and the cecal contents samples of 5 piglets in each treatment were collected and placed in a sterile freezable tube and immediately frozen in liquid nitrogen for the analysis of microbial quantity and microbial metabolites.

**Diet samples and fecal samples**

Representative diet samples of about 2.0 kg were taken from each stage of each treatment group. In addition, representative faeces samples were collected to determine apparent total digestive digestibility of nutrients, according to the method described by Silva et al. (2020) [11]. Simply put, on days 12 to 14 and 26 to 28, fresh faeces were collected from each field using rectal palpation (also known as rectal swab collection), taking care to remove impurities such as pig hair and environmental grit, and the faeces were immediately frozen at −20°C. The feces collected over three days were combined by field, followed by a representative sample of about 400g, which was dried at 65°C for 72 hours. The fecal samples and diet samples were thoroughly crushed and passed through a 40-mesh screen for subsequent nutrient apparent digestibility analysis.

**Determination of growth performance and diarrhea rate**

On the morning of day 1, day 15 and day 29 after weaning, piglets were weighed by head, and the average BW of piglets at day 1, day 14 and day 28 after weaning was counted, and the ADG at day 1–14, day 15–28 and day 1–28 was calculated in the CON and CA group. Feed piglets a few times a day, record the total feed amount according to the field, and record the remaining feed amount in the feed tank at 17:00 every day. The ADFI and FCR (average daily feed intake/average daily gain) of piglets from 1 to 14 days, from 15 to 28 days and from 1 to 28 days were calculated.
During the experiment, the fecal situation of piglets was observed every day, and the anus of piglets was examined one by one to observe whether there was fecal contamination and redness. For fecal score, refer to the scoring criteria of Yuan et al. (1998): 0 point, normal stool; 1 point, paste stool; 2 points, semi-fluid stool; 3 points, liquid feces. If the stool score is \( \geq 2 \), it is considered diarrhea [13]. The proportion of diarrhea and diarrhea frequency of each group was calculated. Proportion of diarrhea (%) = number of diarrhea piglets/number of experimental piglets ×100; Diarrhea frequency (%) = (number of diarrhea piglets × number of diarrhea days)/total number of experimental piglets × number of experimental days ×100.

**Apparent digestibility of nutrients**

Use of indigestible hydrochloric insoluble ash (AIA) in diet and fecal samples as an endogenous indicator [14, 15] to determine the apparent digestibility of dry matter (DM), crude protein (CP) and crude fat (EE). Fecal samples and experimental diets were analyzed for DM (method 930.15), ash (method 942.05), CP (method 990.03) and EE (method 996.01) according to the Official Association of Analytical Chemists (AOAC, 2007). GE was determined by adiabatic bomb calorimeter (Parr 1281, automatic energy analyzer; Moline, IL, USA). Organic matter (OM) is calculated as DM minus ash. AIA concentrations in diet and fecal samples were determined according to the procedure described by Prawirodigidho et al. (2021) [15]. Apparent digestibility of a nutrient (%) = \[1- (\text{content of the nutrient in feces} \times \text{AIA content in diet})/(\text{content of the nutrient in diet} \times \text{AIA content in feces})\]×100.

**Microbial composition in cecal digesta was analyzed by 16S rRNA sequencing**

Total genomic DNA was extracted from each cecal digesta sample using QiAamp Fast DNA stool Minikit (Qiagen, Germany), according to the manufacturer's instructions. The forward primer F (5'-ACTCCTACGGAGGAGGAGCAGCA-3') and the reverse primer R (5'-GGACTACHVGGGTWTCTAAT-3') were used for amplification of the V3-V4 hypervariable region of a 16S rRNA gene. PCR amplicons were purified and quantified, and the PCR products were used for the construction of the libraries and then paired-end sequenced on an MiSeq platform (Illumina, United States) at the Shanghai Penosen Biotechnology Co., LTD (Shanghai, China). Sequencing data were processed using QIIME 2 (version 2019.4), Alpha-diversity values of each sample were assessed based on the observed OTUs, Chao1, and Shannon index. Beta-diversity measures dependent on weighted-UniFrac distance were calculated using mothur. LDA Effect Size (LEfSe) was conducted to identify bacterial taxa differentially represented between different groups at the genus or higher taxonomy level [16].

**Examination of short-chain fatty acid content in cecal digesta**

The SCFAs concentrations of piglets cecal digesta was analysed by a gas chromatographic method. Specifically, approximately 20 mg of cecal digesta was first homogenised in 1.0 ml of 0.5% phosphoric acid solution, and the entire sample was centrifuged at 12 000×g at 4°C for 10 min. Take 100 µl
supernatant and add it to the corresponding 1.5 mL centrifuge tube, add 500 µl MTBE solvent with inner target and swirl for 3 min. After ultrasound in ice bath for 5 min, and centrifuge at 12 000×g at 4°C for 10 min. The sample was injected into a gas chromatography-triple quadrupole mass spectrometry (GC-MS/MS, 8890–7000D, Agilent, USA) equipped with a DB-FFAP column (30m × 0.25mm × 0.25µm). The GC-MS/MS detection parameters were shown in Table 2, and the contents of acetic acid, butyric acid and valeric acid in cecal chymus were determined.

Detection of intestinal permeability markers and inflammatory factors in serum

Serum diamine oxidase activity (DAO), endotoxin (ET), immunoglobulin A (IgA), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor α (TNF-α) were measured by ELISA. The test kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China), and the test method was strictly operated in accordance with the kit instructions.

Statistical analyses

The data of growth performance indicators, apparent digestibility of nutrients, SCFAs content of cecum digesta and serum biochemical indicators were recorded by Excel and processed. Then SPSS 27.0 software was used to conduct one-way ANOVA and Duncan multiple comparison. Chi-square test was used to compare diarrhea rate indicators among groups. \( P < 0.05 \) indicated a significant difference between groups, and \( P < 0.01 \) indicated a very significant difference between groups.

Results

Growth performance and diarrhea of piglets

As shown in Table 3, there were no significant differences in BW, ADFI and ADG of piglets in CON group and CA group during 1–14d, 15–28d and 1–28d after weaning (\( P > 0.05 \)), but the G:F of piglets in CA group during 15–28d and 1–28d after weaning was significantly higher than that in CON group (\( P < 0.05 \)). Comparing the diarrhea of piglets in CON group and CA group, the proportion and frequency of diarrhea of piglets in CA group were significantly reduced (\( P < 0.05 \)).
Table 3
Growth performance of piglets

<table>
<thead>
<tr>
<th>Items</th>
<th>Con</th>
<th>CA</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1d</td>
<td>7.05</td>
<td>7.03</td>
<td>0.19</td>
<td>0.97</td>
</tr>
<tr>
<td>14d</td>
<td>11.05</td>
<td>11.26</td>
<td>0.42</td>
<td>0.82</td>
</tr>
<tr>
<td>28d</td>
<td>16.63</td>
<td>17.17</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>ADFI, g/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-14d</td>
<td>372.21</td>
<td>384.07</td>
<td>23.67</td>
<td>0.83</td>
</tr>
<tr>
<td>15-28d</td>
<td>596.37</td>
<td>590.17</td>
<td>37.18</td>
<td>0.94</td>
</tr>
<tr>
<td>1-28d</td>
<td>484.29</td>
<td>487.13</td>
<td>28.77</td>
<td>0.97</td>
</tr>
<tr>
<td>ADG, g/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-14d</td>
<td>285.40</td>
<td>301.80</td>
<td>17.51</td>
<td>0.68</td>
</tr>
<tr>
<td>15-28d</td>
<td>398.60</td>
<td>422.20</td>
<td>24.99</td>
<td>0.68</td>
</tr>
<tr>
<td>1-28d</td>
<td>342.00</td>
<td>362.00</td>
<td>20.18</td>
<td>0.67</td>
</tr>
<tr>
<td>G:F, g/g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-14d</td>
<td>0.77</td>
<td>0.79</td>
<td>0.01</td>
<td>0.10</td>
</tr>
<tr>
<td>15-28d</td>
<td>0.67b</td>
<td>0.72a</td>
<td>0.01</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>1-28d</td>
<td>0.71b</td>
<td>0.74b</td>
<td>0.01</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

1BW: body weight; ADG: average daily gain; ADFI: average daily feed intake; FCR, feed conversion ratio.
2SEM: standard error of the mean.
3a,b indicate significant differences between groups, P < 0.05.

Apparent digestibility of piglets

As shown in Fig. 2, when piglets were weaned on different diets, the apparent digestibility of DM, OM and CP of piglets in CA group were significantly higher than that in CON group on the 14d and 28d of the experiment (P < 0.05), while the EE level was not significantly different between the two groups (P > 0.05).

Changes of gut microbiota in piglets
In order to investigate whether the improvement of growth performance and apparent digestibility of nutrients in CA group is related to gut microbiota, 16S ribosomal RNA gene sequencing analysis of cecal digesta of piglets was performed. A total of 1,659,597 high-quality sequencing reads were obtained from 20 samples, ranging from 92,381 to 119,090. Based on 97% species similarity, 35,447 ASVs were obtained from samples of the piglets. The alpha and beta diversities were calculated. The results of alpha-diversity measures (Chao1, Shannon and Simpson index) of bacterial community showed that Chao1 index \( (P = 0.07) \) and Simpson index \( (P = 0.09) \) of piglets in CA group increased on the 28th day of the experiment (Fig. 3A). For beta diversity, on day 28 of the experiment period, the interindividual variation of piglets in CA group was lower than that in CON group \( (P < 0.05, \text{Fig. 3B}) \). Community analysis by Venn diagram showed that on 14 d of the experiment, piglets in CA group had 6179 unique ASVs compared with piglets in CON group (Fig. 3C).Venn diagram community analysis showed that piglets in CA group had 6,179 unique ASVs compared with those in CON group on day 14 of the experiment and piglets in CA group had 6032 unique ASVs compared with those in CON group on day 28 of the experiment (Fig. 3C).

As shown in Fig. 4, the CON and CA group piglets have different gut microflora composition at the phylum (Fig. 4A) and the genus levels (Fig. 4B). Linear discriminant analysis (LDA) effect size analysis was used to determine the key genera in the gut microbiota of the CON and CA group piglets. It was found that \( g_{\text{YRC22}} \) was significantly enriched in CA group on the 14th day of the experiment. On the 28th day of the experiment, \( g_{\text{Treponema}}, g_{\text{Pseudomonas}} \) and \( g_{\text{Lachnobacterium}} \) were significantly enriched in CA group \( (P < 0.05, \text{Wilcoxon rank-sum test; log LDA} > 2) \).

**Content of short-chain fatty acids in cecal digesta of piglets**

Analysis of SCFAs content in cecal digesta of piglets in CON and CA groups showed that isobutyric acid content significantly decreased and valeric acid content significantly increased on day 28 compared with day 14 \( (P < 0.05) \). However, CON and CA groups had no significant effect on the content of SCFAs on days 14 and 28 of the experiment.

**Systemic inflammation and intestinal permeability markers levels of piglets**

The effects of CA group treatment on the levels of systemic inflammatory markers in piglets were shown in Fig. 6A-D. On the 28th day of the experiment, the serum levels of pro-inflammatory factor IL-1\( \beta \) in piglets in CA group were significantly reduced compared with those in CON group \( (P < 0.05) \), but the levels of other inflammatory factors were not affected by diet treatment \( (P > 0.05) \). The results of intestinal permeability markers showed that CA group reduced serum endotoxin (ET) content on the 14th day of the experiment.

**Discussion**
Based on the synergistic antibacterial effect of the combination of benzoic acid and 1-monolaurin, we investigated the effects of dietary supplementation of benzoic acid and 1-monolaurin on growth performance, nutrient digestibility and intestinal flora of weaned piglets. The results showed that the simultaneous supplementation of 0.6% benzoic acid and 0.1% 1-monolaurin increased the feed conversion efficiency of piglets at 15–28 days and 1–28 days after weaning, and decreased the proportion and frequency of diarrhea within 14 days after weaning. As no studies have been conducted on the supplementation of benzoic acid and 1-monolaurin in the diets of weaned piglets, we summarized the studies on the supplementation of benzoic acid or glycerol monolaurate in the diets of weaned piglets in combination with other nutrients, and found that 0.35%, 0.5% and 1% benzoic acid supplementation can significantly improve the ADG or G:F of weaned piglets [17–19]. BW, ADG, and relative growth rate were increased when 0.75% glycerol monolaurate and oregano oil were supplemented in broiler diets [20]. These results indicate that benzoic acid and 1-monolaurin supplementation can improve the growth performance of weaned piglets reasonably. Importantly, in our preliminary experiment, we tried the effect of benzoic acid and 1-monolaurin supplementation alone and combined use on diarrhea of weaned piglets, and found that the combined use of benzoic acid and 1-monolaurin further reduced the proportion and frequency of diarrhea of piglets compared with the use of the two alone.

Organic acids are considered an attractive alternative to improve nutrient digestibility in the pig and poultry industry. The multifunctional effects of organic acids include lowering gastric pH, increasing gastric retention time, stimulating pancreatic secretions, affecting mucosal morphology, and acting as substrates in intermediate metabolism, all contributing to improved digestion and absorption [21]. In our study, dietary supplementation with benzoic acid and 1-monolaurin increased the apparent digestibility of dry matter, organic matter and crude protein in weaned piglets for 28 days after weaning. Studies have shown that dietary benzoic acid supplementation significantly reduces the pH value of chyme in stomach and jejunum, increases the concentrations of trypsin, lipase and branchchain protein, and increased the villus height and/or decrease the mucosal crypt depth of duodenum, jejunum and/or ileum of piglets [22–24]. This may be the main reason why benzoic acid and 1-monolaurin supplementation improved nutrient digestibility.

We analyzed whether gut microflora and short-chain fatty acid levels were involved in the regulation of the improvement of growth performance and nutrient apparent digestibility of weaned piglets by dietary supplementation of benzoic acid and 1-monolaurin. The results showed that benzoic acid and 1-monolaurin supplementation significantly changed the composition of gut microflora in caecum contents of piglets. On the 14th day, \( g\_YRC22 \) was significantly enriched in CA group. Summary of previous studies, it has been found that \( g\_YRC22 \) is almost undetectable in the stage of conservation and lactation, but it can rapidly colonize the intestines of growing-finishing pigs and become a dominant bacterium [25]. Benzoic acid and 1-monolaurin supplementation can rapidly enrich \( g\_YRC22 \) in weaned piglets within 14 days after weaning, suggesting that this diet is conducive to intestinal flora stability and rapid colonization of dominant bacteria. In addition, surprisingly, on the 28th day of the experiment, conditional pathogenic bacteria \( g\_Treponema \) and \( g\_Pseudomonas \) were enriched with beneficial bacteria \( g\_Lachnobacterium \) at the same time, which may be related to the large variation among the
piglets in the control group at this stage. In addition, there was no significant difference in the content of short-chain fatty acids in cecal digesta of piglets between the control diet and the experimental diet. These results suggest that benzoic acid and 1-monolaurin treatment may help stabilize the microflora fluctuation caused by weaning of piglets, but have no significant effect on the proliferation of beneficial bacteria and the increase of short-chain fatty acid level.

Weaning stress activates the intestinal immune system and produces a large number of pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6, and IL-8. Overproduction of these cytokines can lead to intestinal damage and dysfunction [26]. Our study found that dietary supplementation with benzoic acid and 1-monolaurin reduced serum IL-1β levels and serum ET levels in weaned piglets. This may be related to MLG-1’s ability to enhance viral inhibitory activity and reduce the release of inflammatory factors [27]. In vivo studies have found that GML supplementation alone in piglets reduced intestinal pathological damage and levels of inflammatory factors IL-8 and TNF-α, thereby improving growth performance [28]. The above information indicates that dietary supplementation of benzoic acid and 1-monolaurin reduced systemic inflammatory response and improve intestinal permeability of weaned piglets, which may be an important reason for improving growth performance of weaned piglets.

**Conclusion**

In conclusion, dietary supplementation of 0.6% benzoic acid and 0.1% 1-monolaurin improved the growth performance of weaned piglets and reduced the rate of diarrhea after weaning. This effect may be related to the increase of nutrient digestibility, the decrease of systemic inflammation and intestinal permeability, and the stability of gut microflora in piglets.

**Declarations**

**Acknowledgments**

The author acknowledged Huanggang Hailin Breeding Leisure Farm Co., Ltd. provides a site for piglet feeding experiments.

**Authors’ contributions**

Kai Wei and Weicheng Bei conceived the study. Xia Yang conducted data collection and laboratory analysis. Kai Wei, Huasheng Zhao and Huanchun Chen participated in the discussion of the results. Kai Wei wrote the manuscript. All authors reviewed the manuscript.

**Funding**

This work was supported by the National Natural Science Foundation of China (32273039), National Key Research and Development Program of China (2021YFD1800400), National Key Research and Development Program of China (2022YFD1800905).
Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All animal research procedures were conducted in accordance with animal research guidelines issued by the Institutional Animal Care and Use Committee of Huazhong Agricultural University.

Consent for publication

Not applicable

Competing interests

None

References


Table 1 and 2

Table 1 and 2 are not available with this version

Figures
Figure 1

Diarrhea of piglets during 1-14 d after weaning. Proportion of diarrhea (A) and diarrhea frequency (B) of weaned piglets. Values were expressed as mean±SEM. *indicates significant difference between CA group and CON group, *P < 0.05.

Figure 2
Apparent digestibility of piglets at 14 d and 28 d of the experiment. Apparent digestibility of dry matter (A), organic matter (B), crude protein (C), and ether extract (D) of piglets at 14 d and 28 d of the experiment. Values were expressed as mean±SEM. *indicates significant difference between CA group and CON group, $P < 0.05$.

**Figure 3**

Gut microbiota composition of piglets on 14 d and 28 d of the experiment. (A) Boxplots of observed operational taxonomic units (OTUs) Chao 1 index, Shannon index, and Simpson index for the CON and CA groups piglets at 14 d and 28 d. Boxes show the medians and 10-90 percentile ranges. (B) The Bray-
Curtis distance between samples of piglets in CON and CA group at 14 d and 28 d of the experiment was analyzed by Principal coordinate analysis. Interindividual variations were determined by average Bray-Curtis distance between individuals in the CON and CA group piglets. Intraindividual variations were determined by distance-paired CON and CA group piglets. *$P < 0.05$. (C) Wein diagram of gut microbiota composition of piglets in CON and CA groups on day 14 and 28 of the experiment.

Figure 4

Linear discriminant analysis (LDA) score for discriminated genera in different groups of piglets at 14 d and 28 d of the experiment. Barplot of relative abundance at the phylum level (A) and genus level (B) for the CON and CA groups. The y-axis represents the relative abundance of each phylum for the two groups. LDA score for discriminated genera of piglets in the CON and CA groups on day 14 (C) and 28 (D) of the experiment. The LDA score is calculated by LDA Effect Size (LEfSe). The value suggests that it is increased in the two groups ($P < 0.05$, Wilcoxon rank-sum test, LDA > 2).
Figure 5

Content of SCFAs in cecal digesta of piglets at 14 d and 28 d of the experiment. Values were expressed as mean±SEM. a,b indicates significant difference between groups, $P < 0.05$.

Figure 6

Intestinal permeability and systemic inflammation markers levels of piglets at 14 d and 28 d of the experiment. Serum (A) IL-1$\beta$, (B) IL-6, (C) IL-8, (D) TNF-\(\alpha\), (E) diamine oxidase (DAO), (F) endotoxin (ET) and (G) immune globulin A (IgA) level of piglets at 14 d and 28 d of the experiment. Values were expressed as mean±SEM. *indicates significant difference between CA group and CON group, $P < 0.05$.

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterial.docx