Analysis of Deoxynivalenol Effects on Mouse Gut Microbiota Using Illumina-MiSeq High-Throughput Sequencing Technology: Postprint

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Abstract

This study aimed to investigate the effects of deoxynivalenol (DON) on gut microbial diversity and species abundance in mice. Forty BALB/c mice with an average body weight of 20 g were selected and randomly divided into a control group (intragastric administration of sterile physiological saline) and a DON group (intragastric administration of 1.8 mg/kg BW DON), with 20 replicates per group (one mouse per replicate), for 28 consecutive days. At the end of the experiment, fresh feces were collected, three samples were randomly selected from each group, and Illumina-MiSeq high-throughput sequencing technology was used to analyze changes in microbial community structure and composition. The results showed that: 1) Compared with the control group, the number of sequences in the DON group decreased ($P > 0.05$). 2) The DON group exhibited decreased Alpha and Beta diversity, and the Shannon index was significantly lower than that of the control group ($P < 0.05$). 3) At the phylum level, compared with the control group, the DON group significantly decreased the species abundance of Bacteroidetes and Deferribacteres ($P < 0.05$), and significantly increased the species abundance of Proteobacteria ($P < 0.05$). At the genus level, compared with the control group, the DON group significantly decreased the species abundance of Parabacteroides, Rikenella, Algoriphagus, Mucispirillum, Methylophilus, and Francisella ($P < 0.05$), and significantly increased the species abundance of Clostridium, Robinsoniella, Allobaculum, and Akkermansia ($P < 0.05$). 4) Cluster analysis showed reduced similarity in gut microbiota between the DON group and the control group. These results indicate that DON can significantly affect the diversity and species abundance of gut microbiota in mice, suggesting that DON-induced intestinal injury is closely related to these changes in gut microbiota.

Full Text

Effects of Deoxynivalenol on Intestinal Microbiota of Mice Analyzed by Illumina-MiSeq High-Throughput Sequencing Technology

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Abstract

This study investigated the effects of deoxynivalenol (DON) on the diversity and species abundance of intestinal microbiota in mice. Forty BALB/c mice with an average body weight of 20 g were randomly divided into a control group (intragastric administration of sterilized saline) and a DON group (intragastric administration of 1.8 mg/kg BW DON), with 20 replicates per group and one mouse per replicate. The administration continued for 28 days. After the experiment, fresh fecal samples were collected, and three samples from each group were randomly selected for analysis of microbial community structure and composition using Illumina-MiSeq high-throughput sequencing technology. The results showed that: 1) Compared with the control group, the number of sequences in the DON group decreased ($P>0.05$). 2) The DON group exhibited reduced Alpha and Beta diversity, with the Shannon index significantly lower than that of the control group ($P<0.05$). 3) At the phylum level, compared with the control group, the DON group significantly decreased the abundance of Bacteroidetes and Deferribacteres ($P<0.05$) and significantly increased the abundance of Proteobacteria ($P<0.05$). At the genus level, compared with the control group, the DON group significantly decreased the abundance of Parabacteroides, Rikenella, Algoriphagus, Mucispirillum, Methylophilus, and Francisella ($P<0.05$), and significantly increased the abundance of Clostridium, Robinsoniella, Allobaculum, and Akkermansia ($P<0.05$). 4) Cluster analysis revealed reduced similarity in intestinal microbiota between the DON and control groups. These findings demonstrate that DON can significantly affect the diversity and species abundance of intestinal microbiota in mice, suggesting that DON-induced intestinal injury is closely related to these alterations in gut bacterial communities.

Keywords: high-throughput sequencing; deoxynivalenol; intestinal microbiota

Introduction

Deoxynivalenol (DON) is a secondary metabolite produced by *Fusarium graminearum* and *Fusarium culmorum* under suitable temperature and humidity conditions [1], predominantly contaminating wheat and corn-based food and feed [2]. Although DON is considered less toxic than other mycotoxins, its widespread

contamination and high concentrations poses significant hazards to humans and animals [3]. Ingestion of DON can readily cause anorexia, vomiting, abdominal pain, diarrhea, fever, unsteady standing, and slowed reactions, while long-term exposure may lead to intestinal dysfunction, growth retardation, immune and nervous system damage, and even shock and death [4-5], resulting in substantial economic losses to animal husbandry.

As the first barrier between the body and external substances, the intestine not only prevents invasion by xenobiotics such as feed proteins, natural toxins, microorganisms, and mycotoxins, but also serves as a vulnerable target for these agents. Recent research has focused on DON' s effects on intestinal function, revealing that DON can damage intestinal mucosa, disrupt intestinal barrier function and tight junctions, induce intestinal inflammation, reduce intestinal immune response, and hinder nutrient absorption [6-7].

Intestinal microbiota, regarded as a "black box" of the organism, is an essential component of the gut. All its life activities and metabolic processes, including barrier function, nutritional roles, and immune response, are closely related to health [8]. However, studies on DON' s effects on intestinal microorganisms are scarce both domestically and internationally. In vitro experiments have shown that DON does not affect the growth of *Staphylococcus aureus*, *Escherichia coli*, and *Yersinia enterocolitica*, but only inhibits *Streptococcus* [9]. Waché et al. [10] reported that feeding pigs DON-contaminated diets increased anaerobic bacteria while decreasing anaerobic sulfite-reducing bacteria. Saint-Cyr et al. [11] found that intragastric administration of DON in mice significantly reduced fecal *E. coli* counts. However, these studies were based on culture methods, capillary electrophoresis-single strand conformation polymorphism (CE-SSCP), and quantitative PCR, which cannot fully reflect DON' s effects on intestinal microbiota. They were limited to bacteria of certain abundances or specific microorganisms, with unknown microbes not being addressed. Therefore, this study employed high-throughput, deep-detection, and high-accuracy metagenomic sequencing technology combined with bioinformatics analysis to qualitatively and quantitatively investigate DON' s effects on the structural composition, diversity, and abundance of mouse intestinal microbiota. The aim was to explore the mechanisms underlying DON-induced intestinal inflammation, damage, and nutrient absorption impairment, identify sensitive bacterial groups and microbial markers for intestinal DON exposure, and explore new approaches for safety evaluation of mycotoxin contamination in feed.

1.1 Materials and Instruments

DON standard: powder form, purity $ 99×$Taq PCR Master Mix were purchased from Tiangen Biotech (Beijing) Co., Ltd.

Main instruments: Illumina-MiSeq DNA sequencer (Illumina, USA), UV spectrophotometer (Shimadzu, Japan), Sorvall high-speed centrifuge (Thermo, USA), PCR instrument (Bio-Rad, USA), and gel imaging system (Bio-Rad,

USA).

1.2 Experimental Animals and Management

Forty BALB/c mice, 6 weeks old, weighing 18–22 g, specific pathogen-free (SPF) grade, were purchased from the Laboratory Animal Science Department of Fudan University. Room temperature was maintained at (23$±$2)°C, relative humidity at 40%–70%, with a 12-hour light cycle. Mice had free access to water and feed, which consisted of autoclaved distilled water and feed sterilized by 1.5–2.5 Mrad cobalt-60 irradiation. The mice were acclimated for one week before the experiment.

1.3.1 Experimental Design

The 40 mice were randomly divided into 2 groups with 20 replicates per group and one mouse per replicate, housed separately in sterile isolators. The groups consisted of a control group (intragastric administration of sterilized saline) and a DON group (intragastric administration of 1.8 mg/kg BW DON). The dose was designed based on acute and subacute toxicity results of DON in BALB/c mice [15]. Each mouse received 0.2 mL per day, and the experiment lasted for 28 days.

1.3.2 Sample Collection

After the experiment, mice were immobilized, their tails lifted, and their lower abdomens gently pressed to collect fresh fecal samples, which were placed in 1.5 mL autoclaved centrifuge tubes and stored at -80°C.

1.3.3 Fecal DNA Extraction and Quality Control

Three fecal samples were selected from each group. Exactly 0.2 g of each sample was weighed into 2 mL centrifuge tubes, and DNA was extracted using a fecal DNA extraction kit. DNA concentration was measured using a UV spectrophotometer, and purity was assessed by 0.8% agarose gel electrophoresis. DNA samples were then sent to Shanghai Paisheng Nuo Biotechnology Co., Ltd. for sequencing analysis.

1.3.4 Illumina-MiSeq Metagenomic Sequencing

1.3.4.1 16S rDNA V4 Region PCR Reaction

Universal primers were designed based on the conserved V4 region sequence of the 16S rDNA gene: forward primer 5’ -AYTGGGYDTAAAGNG-3’ and reverse primer 5’ -TACNVGGGTATCTAATCC-3’ . To distinguish different samples, a 7-nucleotide barcode sequence (Barcoded-tag) was randomly added to the upstream sequence of the forward primer to form a barcoded fusion primer. The PCR reaction system consisted of: Loading Buffer (10×) 2.5 L, dNTP 2 L, DNA template 1 L, forward primer 1 L, reverse primer 1 L, DNA polymerase

0.125 L, and ddH_2O 17.375 L. PCR conditions were: pre-denaturation at 94°C for 5 min; 25 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 30 s; final extension at 72°C for 7 min; storage at 4°C. Products were detected by 2% agarose gel electrophoresis, target bands were recovered using a gel extraction kit, and sample concentrations were measured using a BioTek microplate reader for quantification.

1.3.4.2 Enrichment and Amplification of DNA Libraries

DNA from each sample was mixed in equal amounts, and the required metagenomic sequencing library was constructed using the standard Illumina TruSeq DNA library preparation protocol. Barcoded Illumina MiSeq sequencing was performed on the Illumina-MiSeq Personal Genome Analysis Platform using the PE250 strategy. The workflow involved: repairing DNA fragments with overhangs using 3' -5' exonuclease and polymerase, introducing a single base A at the 3' end of the repaired fragments, and ligating adapters with a single base T at the 3' end to ensure A-T pairing. PCR was used to selectively enrich DNA fragments with adapters at both ends and amplify the DNA library.

1.3.4.3 Library Validation and Sequencing

Libraries were quantified using PicoGreen and a fluorescence spectrophotometer, normalized to 10 nmol/L for multi-sample DNA libraries, mixed in equal volumes, and gradually diluted to 4–5 pmol/L for sequencing.

1.4.1 Basic Sequencing Data Processing

Quality control was performed on paired-end sequencing data by truncating or discarding low-quality sequences (50 consecutive bases with average quality >Q30, no N allowed). Sequences passing quality control were joined using Flash software, and unjoinable sequences were discarded. Filtered sequences were further processed by discarding sequences with lengths outside 200–1000 bp, containing ambiguous bases, containing mismatched bases, having >6 consecutive identical bases, or >1 ambiguous base, yielding final sequences for analysis.

1.4.2 Taxonomic Classification and Statistical Analysis

Qiime software was used to cluster sequences into operational taxonomic units (OTUs) based on sequence similarity. Qiime called Uclust for sequence clustering, and the longest sequence in each cluster was selected as the representative sequence. RDP-classifier was used to annotate OTU representative sequences using the RDP database as a training set.

Species abundance was calculated at the genus level. Data were normalized by $\log_2$ transformation, followed by subtraction of the single-sample abundance mean and division by the single-sample abundance standard deviation. Data were imported into SPSS 19 for statistical analysis using ANOVA, t-tests, and LSD multiple comparison tests, with $P<0.05$ considered statistically significant.

2.1 Effects of DON on Intestinal Microbial Sequencing Data in Mice

As shown in Table 1 , after Illumina MiSeq paired-end sequencing of the 16S rDNA V4 region, the control and DON groups obtained 86,146.23 and 84,594.98 effective sequences, respectively, with 85,843.64 and 84,305.31 high-quality sequences. Although the DON group showed reduced sequence numbers compared with the control group, the difference was not statistically significant (P>0.05). The rarefaction curve reflecting sampling depth at 97% similarity is shown in Figure 1 [Figure 1: see original paper], with each sample reaching approximately 80,000 reads, indicating consistent sampling depth. Sequence lengths ranged from 178–268 bp, with an average length of 226 bp (Figure 2 [Figure 2: see original paper]).

Table 1 Analysis of sequencing quantities for fecal samples of mice

Items	Control group	DON group	P-value
Effective sequences	86,146.23$\pm$11, 856.48\|84, 594.98$\pm$7, 317.08\|\|\|*Highqualitysequences*\|85, 843.64$\pm$11, 831.43|84, 30		

In the same row, values with different small letter superscripts mean significant difference (P<0.05), while with the same or no letter superscripts mean no significant difference (P>0.05). The same as below.

C1, C2, C3 indicates the sample 1, sample 2 and sample 3 in control group; T1, T2, T3 indicates the sample 1, sample 2 and sample 3 in DON group. The same as below.

2.2 Abundance Distribution Curves of Intestinal Microbiota in Mice

OTU abundance distribution curves were generated by ranking OTU abundance in each sample and plotting $\log_2$ values (Figure 3 [Figure 3: see original paper]). The results showed uniform species distribution in both control and DON group samples, suitable for diversity analysis.

2.3 Effects of DON on Alpha Diversity of Intestinal Microbiota in Mice

All sequences from the two groups were clustered into OTUs at 97% similarity, and the Shannon index was calculated to evaluate microbial alpha diversity richness and evenness (Figure 4 [Figure 4: see original paper]). The Shannon index in the DON group was significantly reduced compared with the control group (P<0.05).

Figure 4 Change of Shannon diversity indexes of intestinal microbes exposed to DON

- indicates significant difference compared with control groups (P<0.05).

2.4 Effects of DON on Beta Diversity of Intestinal Microbiota in Mice

Beta diversity compares diversity between different ecosystems and represents the rate of species composition change along environmental gradients or among communities, reflecting biotic responses to environmental heterogeneity. Principal coordinates analysis (PCoA) revealed clear separation between the DON and control groups, with the DON group showing compact distribution and high similarity in community structure (blue), indicating reduced diversity compared with the control group (Figure 5 [Figure 5: see original paper]).

Figure 5 Change of beta diversity of intestinal microbes exposed to DON

2.5 Differential Analysis of Species Abundance

To investigate DON' s effects on intestinal microbial community structure and composition, OTUs were clustered and compared at both phylum and genus levels. As shown in Table 2 and Figure 4, at the phylum level, the abundance of Bacteroidetes was significantly decreased in the DON group compared with the control group (P<0.05), with its proportion in the total community decreasing from 45.01% to 35.25%. The abundance of Deferribacteres was also significantly decreased (P<0.05), with its proportion decreasing from 0.76% to 0.39%. The abundance of Tenericutes showed a decreasing trend but was not significantly different (P>0.05). The abundance of Proteobacteria was significantly increased (P<0.05), with its proportion rising from 6.66% to 8.78%. The abundance of Verrucomicrobia showed an increasing trend but was not significantly different (P>0.05). Firmicutes accounted for 43.05% of the total community in the control group and increased to 50.41% in the DON group, but this difference was not statistically significant (P>0.05).

Figure 4 The pie chart of intestinal microbes distributed on phylum level

Table 2 Phylum level significance analysis of intestinal microbes

Phylum	Control group	DON group	Relative fold change/[Log(C/T,2)]	P-value
Bacteroidetes	38,534.20$±8,510.69^a	28,922.44±1,495.53^b	< 0.01	
Deferribacteres	555.45±46.06^a	378.73±95.61^b	< 0.01	
Proteobacteria	5,294.64±587.99^b	6,441.51±841.95^a	< 0.01	
Tenericutes	234.10±80.28	148.46±16.54		
Verrucomicrobia	680.95±35.77	2,472.37±$474.48		

C represents the control group, T represents the DON group. The same as below.

Table 3 shows that at the genus level, compared with the control group, the DON group significantly decreased the abundance of Parabacteroides, Rikenella, Algoriphagus, Mucispirillum, Methylophilus, and Francisella ($P<0.05$), and significantly increased the abundance of Clostridium, Robinsoniella, Allobaculum, and Akkermansia ($P<0.05$). Additionally, DON treatment affected the abundance of Brevibacterium, Coprococcus, Kordiimonas, Pusillimonas, and Anaeroplasma, but these differences were not significant compared with the control group ($P>0.05$).

Furthermore, Figure 6 [Figure 6: see original paper] presents a heatmap showing inter-species similarity clustering relationships at the genus level. The results demonstrated that DON treatment altered the intestinal microbial structure in mice, with significantly different similarity compared with the control group, consistent with the results in Table 3.

Table 3 Genus level significance analysis of intestinal microbes

Genus	Control group	DON group	Relative fold change/[Log(C/T,2)]	P-value
Brevibacterium	0.63±0.56	1.25±0.48		
Parabacteroides	1,806.26±607.93^a	937.05±281.09^b		< 0.01
Rikenella	550.72±90.21^a	323.72±14.15^b		< 0.01
Algoriphagus	3.90±1.61^a	1.30±1.08^b		
Mucispirillum	555.45±46.06^a	378.73±95.61^b		< 0.01
Clostridium	0.01±0.00^b	1.41±0.69^a	0.36±0.14	0.83±0.09
Coprococcus	121.38±60.49^b	390.96±2		
Akkermansia				

Figure 6 Change of bacterial distribution of intestinal microbes exposed to DON

The animal intestine harbors a large number of microbial communities that maintain dynamic equilibrium with the host and specific proportional relationships among different bacterial groups. Microbial homeostasis plays a crucial role in maintaining physiological functions such as growth and development, nutrient digestion and absorption, and immune antagonism [12]. This study employed Illumina-MiSeq high-throughput sequencing technology, overcoming limitations of traditional culture methods and 16S rDNA-based molecular biology approaches. This technology generates broad-coverage, deep sequencing data that, through alignment or cluster analysis, can determine changes in microbial community species composition while mining low-abundance or unknown bacteria. This enables more comprehensive and accurate identification of sensitive bacterial groups, which is important for accurately determining the toxic effects of DON on intestinal microbiota.

3.1 Effects of DON on Intestinal Microbial Diversity

Intestinal microbial diversity is fundamental for promoting nutrient absorption and maintaining host immunity and metabolism. Reduced diversity can easily affect host immunity and consequently impact health [13]. This study indicated

that while there was no significant difference in sequencing quantities between the two groups after DON administration, both effective and high-quality sequences in the DON group were reduced compared with the control group, suggesting a decrease in microbial species. Furthermore, the Shannon index, which reflects alpha diversity richness and evenness, was significantly reduced in the DON group. Beta diversity analysis using PCoA revealed compact distribution and reduced diversity of the microbial community structure in the DON group. However, studies by Waché et al. [10] and Piotrowska et al. [14] showed that dietary DON supplementation did not affect the abundance and diversity of pig intestinal microbiota. These discrepancies may be attributed to differences in detection methods, DON dosage, and animal species. Our previous research indicated that intragastric administration of DON reduced the apparent digestibility of intestinal amino acids and mineral elements, decreased mouse body weight, and impaired immune function [15], suggesting these effects may be related to DON-induced reduction in intestinal microbial diversity.

3.2 Effects of DON on Intestinal Microbial Structure and Species Abundance

This study used Illumina-MiSeq sequencing technology to detect changes in microbial community structure and composition in mouse feces. The results revealed significant alterations in species abundance following DON treatment, showing certain regular patterns. The dominant bacterial phyla in the human gut are Bacteroidetes and Firmicutes, accounting for over 90% of the intestinal microbiota, while in mice these two phyla comprise about 85% [16]. In this experiment, Bacteroidetes and Firmicutes accounted for over 80% of mouse intestinal microbiota, and the total proportion of these two dominant phyla remained unchanged after DON treatment, but the abundance of Bacteroidetes was significantly decreased compared with the control group.

Bacteroidetes is a bacterial group that promotes carbohydrate fermentation and participates in the metabolism of carbohydrates, bile acids, and steroids [17]. Wang et al. [18] reported that intragastric administration of aflatoxin B1 in rats significantly inhibited the abundance of Bacteroidetes-related bacteria, and the abundance of Bacteroidetes was also significantly reduced during intestinal inflammation or ulceration [19], consistent with our findings. Additionally, the abundance of Deferribacteres decreased while Proteobacteria increased. In a mouse model of non-alcoholic fatty liver disease induced by high-fat diet, increased abundance of Proteobacteria was accompanied by decreased expression of the tight junction protein occludin, indicating compromised intestinal mucosal integrity and suggesting intestinal injury [20]. These collective results indicate that DON, like aflatoxin and high-fat diet, may alter intestinal microbial abundance, thereby inducing intestinal inflammation and causing intestinal damage.

At the genus level, compared with the control group, the DON group significantly reduced the abundance of Parabacteroides, Rikenella, Algoriphagus, Mu-

cispirillum, Methylophilus, and Francisella. Numerous studies have indicated that reduced abundance of these genera is closely associated with intestinal diseases. Noor et al. [21] reported that patients with ulcerative colitis had lower expression levels of Parabacteroides in fecal microbiota than healthy individuals, suggesting that the absence of this genus may be related to intestinal inflammation. The role of Rikenella in the intestine remains unclear, but studies have suggested that Rikenellaceae is a protective bacterial group in the gut [22]. The significant reduction in Rikenella abundance in this study indicates that DON may inhibit beneficial intestinal bacteria. Mucispirillum serves as a marker for intestinal bacterial communities, with its abundance significantly decreasing during early-stage enteritis or antibiotic application [23], consistent with our finding that DON reduced Mucispirillum abundance. We speculate that intragastric administration of DON in mice may reduce the abundance of inflammation-sensitive bacterial groups such as Parabacteroides and Mucispirillum, as well as beneficial bacteria Rikenella, thereby inducing intestinal inflammation, causing intestinal damage, and harming host health.

Additionally, DON decreased the abundance of Algoriphagus, Methylophilus, and Francisella in the intestine. Since these genera have low proportions in the intestinal microbiota, immature isolation and culture techniques, and limited functional studies, further investigation is warranted.

DON administration significantly increased the abundance of Clostridium, Robinsoniella, Allobaculum, and Akkermansia. Many studies have shown that changes in these genera are also closely related to intestinal infection and inflammation. *Clostridium*, a genus within Firmicutes, includes various pathogenic bacteria such as *Clostridium perfringens* and *Clostridium botulinum* that produce exotoxins with strong toxic effects on humans and animals. Piotrowska et al. [14] found that feeding pigs DON-contaminated diets increased *Clostridium perfringens* content in colonic contents, suggesting that DON can induce increased pathogenic bacteria in the intestine. *Robinsoniella* is considered a secondary invader in clinical intestinal infections and can be isolated from wound infection sites or the blood of bacteremia patients [24]. In an IL-10 knockout mouse model of enteritis, the abundance of *Robinsoniella peoriensis* was significantly increased [25]. The increased abundance of Robinsoniella in our study suggests that DON may induce intestinal inflammation and infection. Although Allobaculum has a low proportion in intestinal microbiota, studies have shown that its abundance increased in mice with enteritis induced by high-cholesterol diet, and its abundance was negatively correlated with anti-inflammatory factor expression [26]. Zhou et al. [27] reported that intragastric administration of gutter oil in mice caused intestinal mucosal damage and induced enteritis, with Allobaculum abundance also significantly increased, consistent with our DON-induced increase in Allobaculum abundance.

Akkermansia is a hot research topic in recent years. As the dominant genus of Verrucomicrobia, it accounts for about 83% of this phylum and 1–4% of total intestinal microbiota [28], utilizing intestinal epithelial mucin as carbon

and nitrogen sources and capable of reducing intestinal mucus barrier thickness [29-30]. Multiple studies have found that Akkermansia abundance is reduced in overweight or obese individuals, suggesting that it may reduce fat deposition and body weight [31-32]. Yang et al. [15] found that intragastric administration of DON significantly reduced mouse body weight. Additionally, Akkermansia can disrupt host mucosal homeostasis and exacerbate intestinal inflammation induced by *Salmonella typhimurium* [33]. We infer that DON-induced body weight reduction and intestinal inflammation in mice may be related to increased Akkermansia abundance.

To date, numerous reports have documented DON-induced intestinal inflammation, focusing primarily on reduced intestinal villus height, decreased crypt depth, and increased expression of inflammatory cytokines IL-12 and TNF-α in intestinal epithelial cells [34-35]. However, these studies did not address changes in intestinal microbial communities. Whether DON-induced alterations in intestinal microbial abundance are directly related to intestinal inflammation requires further verification. Given the correlation between reduced abundance of Parabacteroides and Mucispirillum and increased abundance of Clostridium, Robinsoniella, Allobaculum, and Akkermansia with intestinal infection and inflammation, changes in the abundance of these bacterial groups may serve as microbial markers for DON-induced intestinal inflammation and compromised gut health.

4 Conclusions

1. After intragastric administration of DON, the abundance of intestinal microbiota in mice decreased, with reduced Alpha and Beta diversity.
2. At the phylum level, compared with the control group, the DON group significantly decreased the abundance of Bacteroidetes and Deferribacteres and significantly increased the abundance of Proteobacteria. At the genus level, the DON group significantly decreased the abundance of Parabacteroides, Rikenella, and Mucispirillum, and significantly increased the abundance of Clostridium, Robinsoniella, Allobaculum, and Akkermansia.
3. Changes in the abundance of these bacterial groups in the intestine are closely related to intestinal inflammation, suggesting they may serve as microbial markers for DON-induced intestinal inflammation.

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Note: Figure translations are in progress. See original paper for figures.