DOI: 10.26599/FSHW.2026.9250997

Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: https://www.sciopen.com/journal/2097-0765

China Food Publishing Co.

Persistence and Dynamics of *Lactiplantibacillus plantarum* in the Gut and Its Impact on the Healthy Host

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ABSTRACT: This study investigated the colonization site and duration of L. plantarum CCFM8610 in the host intestine using in vivo and ex vivo fluorescence imaging. The results indicated that orally administered CCFM8610 primarily remained in the cecum and colon and could transiently survive 6 days in the intestine after cessation of administration. The impact of CCFM8610 on gut microbiota was described by 16s rRNA sequencing comparison of mouse and human fecal samples. The results showed that the intake of CCFM8610 would change the gut microbes at the phylum level, including an increase in the abundance of Firmicutes and a decrease in Fusobacteria, and some changes were persistent. Furthermore, based on functional predictions and metabolic results, ingestion of CCFM8610 primarily affects lipid metabolism pathways in healthy hosts especially bile acid metabolism. Overall, this study investigated the colonization ability of CCFM8610 in healthy hosts after ingestion and its effects on the host, providing an effective reference for the study of daily intake regimens of probiotics.

Keywords: *L. pantarum*; colonization; metabolite; bile acid

1. Introduction

Probiotics confer health benefits when administered in sufficient doses to the host [1]. Among these, *Lactobacillus* spp. are the most widely used microorganism[2]. They are widely distributed in the environment and various foods (cheese, yoghurt, and sausage)[3]. In addition, *Lactobacillus* spp. can colonize multiple sites in the human body, including the entire digestive tract (from the mouth to the feces), skin, and vagina[4].

As a member of the genus *Lactobacillus*, *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) can survive in a variety of environments[5-7]. However, compared to *Limosilactobacillus reuteri* and *Ligilactobacillus salivarius*, which can colonize the host, *L. plantarum* is unable to remain in the host intestinal tract for a long time and is only a passing bacterium in the intestinal tract[8, 9]. Nevertheless, they can still exert significant probiotic functions in the intestinal tract, including maintaining the balance of the gut microbiota, improving host immunity, ameliorating intestinal inflammation, antifungal properties, alleviating

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Received 18 July 2025
Received in revised from 20 August 2025
Accepted 4 November 2025

heavy metal toxicity, lowering cholesterol levels, and alleviating lactose intolerance, etc[10-13]. This suggests that although *L. plantarum* cannot survive in the host intestine for a long time, it still plays a probiotic role in the host intestine. Therefore, it is important to investigate how *L. plantarum* exerts its probiotic function during short-term intake and whether it can exert a modulatory effect on the host gut microbiota over a longer period.

To better understand the survival, metabolic activities, and bacterial-flora interactions of *L. plantarum* in the gastrointestinal tract (GIT), we selected CCFM8610 as the model strain for analysis. As a typical strain of *L. plantarum*, CCFM8610 (isolated from fermented vegetables) has been extensively studied. It is facultatively anaerobic and well-tolerated in the gastrointestinal environment. In addition, it exhibits good antioxidative ability[14]. Indeed, in mouse models of acute and chronic cadmium (Cd) poisoning, ingestion of CCFM8610 effectively decreased the intestinal absorption of Cd, alleviated its accumulation in tissues, and reversed renal and hepatic damage[15, 16]. Moreover, in a murine model of ulcerative colitis induced by DSS, CCFM8610 can significantly alleviate colitis[17]. Finally, several other probiotic functions have been identified for this strain[18, 19]. Collectively, these results suggest that CCFM8610 is an excellent probiotic strain for use as a model in this study.

In the present study, using CCFM8610 as a model bacterium, we studied its survival, distribution, and duration in the GIT of humanized mice using bioluminescence imaging. Moreover, 30 human volunteers took CCFM8610 once for verification and compared it with the results of animal experiments to explain the metabolic activity and bacterial flora interactions of CCFM8610.

2. Material and Methods

2.1 Experimental Models and Study Participants

2.1.1 Bacterial Strains, Plasmids, and Growth Conditions

The *RFP* gene under the control of P23 was cloned into pIB184 as *Bam*HI-*Eco*RI fragment. The resulting plasmid was subsequently transferred into CCFM8610 using electro-transformation and renamed 8610-RFP (Table 1).

L. plantarum strains were grown in Man-Rogosa-Sharpe (MRS) medium at 37 °C. If necessary, lactobacilli were screened by adding erythromycin to the medium at a concentration of 1500 μg/mL.

Table 1 Bacterial strain, plasmid and abbreviation for recombinant strain used in this study

Strain, plasmid, or abbreviation for recombinant strain	Description	Source
Strain: CCFM8610	Originally isolated from fermented vegetables	This study
Plasmid: pIB184-p23-RFP	pIB184 carries RFP cDNA fused to the strong promoter *P23*	This study
Abbreviation for strain: 8610-RFP	CCFM8610 expressing RFP fluorescent gene	This study

2.1.2 Animals

C57Bl/6J mice (male, 4 weeks of age) were purchased from SPF Biotechnology Co., Ltd. (Beijing, China). The animal care and study protocols were approved by the Ethics Committee of Jiangnan University,

China (JN.No20220315c1800630[058]). The mice were housed under a specific pathogen-free (SPF) environment at the Laboratory Animal Center of the Department of Food Science and Technology, Jiangnan University (Wuxi, China) in a temperature- (20–26 °C), humidity- (40–70%), and illuminance- (15–20 LX) controlled room.

2.1.3 Human Participants

This study was approved by the Medical Ethics Committees of Jiangnan University. This study was registered in the Chinese Clinical Trial Registry (clinical trial number: JNU20230601IRB14). All participants provided informed consent prior to sample collection. All participants were aged 18–70 years of age and were not currently following any dietary program or dietitian consultation. Exclusion criteria were as outlined below: use of antibiotics within the past month, acute or chronic gastrointestinal diseases, and other conditions (diabetes mellitus, hypertension, and cancer), having undergone major abdominal surgery, women during pregnancy or lactation, yoghurt, probiotics, prebiotics, synbiotics, and dietary supplement use within the previous month. A total of 60 participants were enrolled in this study.

2.2 Mice Experimental Design

2.2.1 Pseudo Germ-free Modeling and Fecal Microbial Transfer

The intestinal microbiota was first depleted using broad spectrum antibiotics for more than 14 d. Antibiotic treatment was administered according to previous procedures[20]. Fecal samples were collected directly from healthy donors and immediately suspended in ice-cold saline by homogenization at a concentration of 60 mg of feces/mL. The supernatant was collected after incubation on ice for 2 min. Fecal microbiota transplantation (FMT) was administered to mice by gavage of 200 μL of fecal suspension for 5 d, consecutively following the antibiotic intervention.

2.2.2 In vivo Persistence of L. plantarum in the GIT of Humanized Mice

The experimental procedure is shown in Figure 1A. Mice received 5×10^9 culture-forming units (CFU) of live 8610-RFP for 4 d. One group of mice received normal saline as a negative control. Fecal samples were collected daily during gavage and post-administration. Gradient dilutions of the samples were inoculated onto selective MRS medium (1500 μg/mL erythromycin) for counting. Erythromycin-resistant bacteria were not detected in the negative control group. After euthanasia, mouse intestines were resected for *ex vivo* imaging.

2.2.3 In vivo Fluorescence Imaging

Fluorescence imaging was performed using an IVIS Spectrum Imaging System (PerkinElmer). All mice were anesthetized by injecting 1.5% isoflurane in the air using a gas anesthesia system (Tem Sega, Lormont, France). The emitted fluorescence was measured at specific excitation (535 and 570 nm) and emission (600–700 nm) wavelengths. The optimal excitation and emission wavelengths (highest signal in whole-body imaging of mice) were selected to obtain several images with an exposure time of 3 s. The radiative efficiency of the fluorescence was quantified using Living Image software, and pseudocolor images representing the detected light intensity were generated.

2.3 Human experimental Design and Sample Collection

A single dose of CCFM8610 (5×10^9 CFU) was administered to humans to assess its impact on intestinal microbiota. Thirty patients (15 males and 15 females) received a single dose of CCFM8610. Stool samples were collected from all participants at up to four time points (baseline and days 1, 4, and 7). Samples collected were immediately frozen in a -20 °C freezer and delivered to the laboratory on dry ice as rapidly as possible and subsequently stored at -80 °C.

2.4 Fecal Microbiome Sequencing

To determine gut microbiota alterations induced by CCFM8610, DNA was isolated from fecal samples using the Fast DNA Spin Kit (MP Biomedicals Ltd., Santa Ana, CA, USA). The V3 and V4 regions of the 16s rRNA gene were sequenced using the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA). Quality filtering and demultiplexing were performed using the DADA2 package in QIIME2 (v. 2022.2.0). Sequence alignment and taxonomic annotation were performed using the Silva Bacterial Database. We used the R vegan, Mfuzz, and tidyverse libraries for the analysis and visualization.

2.5 Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthologs and Pathways

To determine the effect of CCFM8610 intake on gut microbiota metabolism, we performed functional prediction of gene repertoires using PICRUSt2 (v 2.5.0). The KEGG orthologs (KO). Based on the inferred KEGG metabolic orthologs/pathways, differential metabolic pathways at different time points were assessed using the linear discriminant analysis (LDA) effect size (LEfSe).

2.6 Metabolomic Analysis

Stool samples were mixed with 200 μL ddH_2O. The proteins were precipitated by adding methanol:acetonitrile (1:1). Subsequently, the supernatant was evaporated to dryness at 15000 rpm. Finally, acetonitrile:water (1:1) was used to redissolve the liquid, which was then subjected to ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). The detection parameters were identical to those used by Wang et al[21].

Raw metabolomics data were processed using Compound Discover v3.3 (Thermo fisher Scientific, USA). Potentially differential metabolites were searched using variable importance (VIP) projections (SIMCA v. 14.1, Germany), and p-values were calculated using the Wilcoxon test.

2.7 Cholic Acid Targeted Metabolome Analysis

Target bile acids in fecal samples were quantified using liquid chromatography-tandem mass spectrometry. For sample preparation, 20 mg of sample was homogenized with 200 μL of water. 800 μL of methanol: acetonitrile (1:1, v/v) was then added. The sample was vortexed and sonicated on ice. The sample was incubated at -20°C for 1 hour and then centrifuged at 15,000 rpm for 15 minutes at 4°C. The sample was filtered through a 0.22 μm filter and stored in a vial for analysis by liquid chromatography-tandem mass spectrometry. Appropriate amounts of bile acid standards were weighed to prepare individual standard stock

solutions. Appropriate amounts of each stock solution were then used to prepare mixed standards, which were diluted sequentially with methanol to the appropriate concentrations to prepare working standard solutions.

The liquid chromatography was performed on a Thermo U3000 using an ACQUITY UPLC® HSS T3 1.8 μm (2.1 × 100 mm) column. The mass spectrometry was performed on a Thermo Q Exactive. Specific conditions were based on the methods of Weike Qian et al[22].

2.8 Quantification and Statistical Analysis

Comparisons between two groups were made using the Student's t-test or the Wilcoxon test. The Kruskal-Wallis test was used for multiple group comparisons. All tests were performed using the GraphPad Prism Software (v. 9.0.0). Statistical analysis was performed using R 4.1.3 with the relevant base function. Statistical significance was set at $P < 0.05$. Multiple comparisons were corrected using the Benjamini–Hochberg FDR method, and adjusted p-values were reported.

3. Results

3.1 CCFM8610 can persist for a short time in the intestine of humanized mice

To investigate the colonization ability of CCFM8610 in the mouse intestinal tract deeply, we identified the site of CCFM8610 residence in the mouse intestine using *in vivo* and *ex vivo* fluorescence imaging of intestinal tissues after daily oral intake (Figure 1A). The fluorescence was strong both *in vivo* and *ex vivo* on days 1–4 (during oral intake of the strain). The signals of 8610-RFP were mostly found in the cecum and colon (Figure 1E), suggesting that CCFM8610 is primarily found in the cecum and colon. The fluorescence signal of 8610-RFP in humanized mice decreased rapidly after the cessation of administration (days 5–7, Figure 1B). This result was confirmed by our *ex vivo* fluorescence results of tissues, where the 8610-RFP signal rapidly decreased to undetectable levels after cessation of administration (days 5–7, Figure 1E).

The fluorescence signals were quantified daily (using fluorescence imaging) for each group of five anesthetized mice daily (Figure 1B). The fluorescence signal was strong in mice after receiving 8610-RFP, with slight variations in signal intensity from day 1 to the last day of 8610-RFP administration (day 4), peaking on day 4. The 8610-RFP signal fell rapidly to near background levels after the cessation of administration. In humanized mice, the 8610-RFP strain persisted for 4 d after the last ingestion. However, the sensitivity of fluorescence imaging is limited, and it is not possible to accurately detect the number of 8610-RFP viable bacteria in the intestines of mice, making it difficult to detect their persistence in the intestines. The residence time of 8610-RFP in the gut was confirmed by 8610-RFP levels in fecal samples (the complete disappearance of 8610-RFP from the intestine was confirmed by fecal culture only) (Figure 1A). 8610-RFP persisted in fecal samples until day 10 (6 d after the last ingestion) and was no longer detectable by day 11 (Figure 1D). Collectively, these results suggest that ingested heterologous CCFM8610 is capable of short-term residence at the end of the host intestine but is unable to colonize the host GIT on a long-term basis.

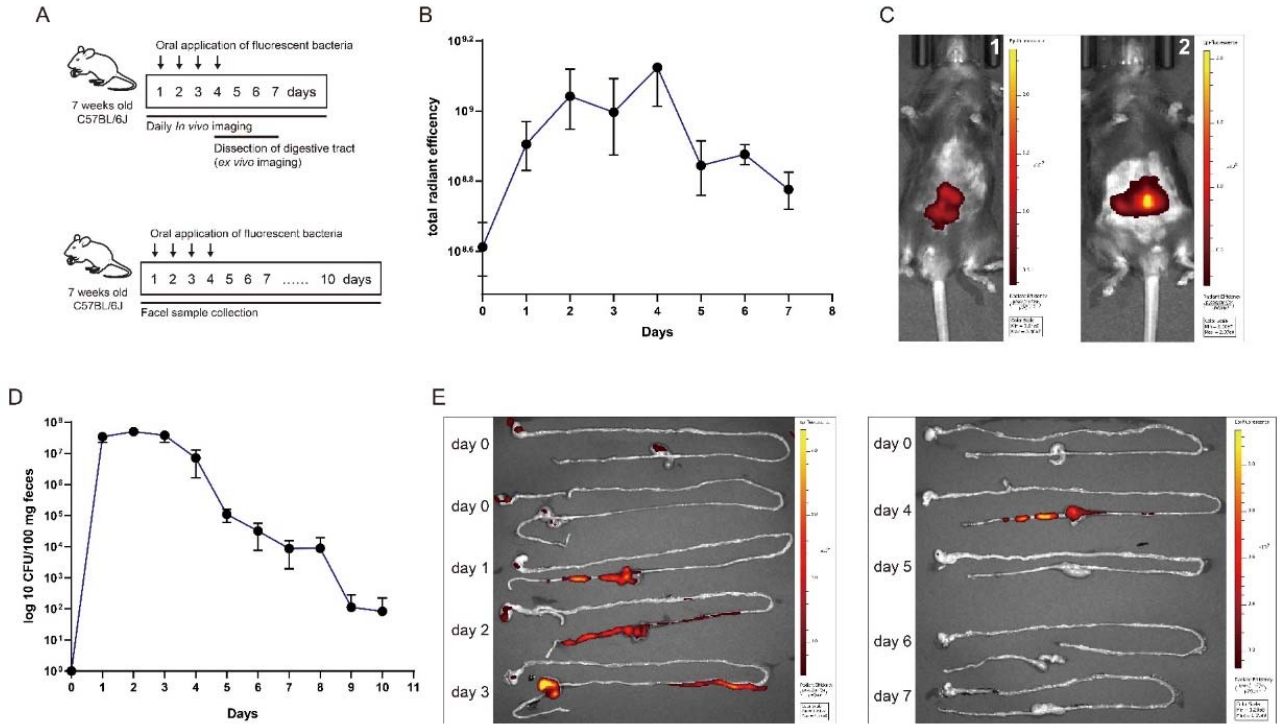


Figure 1. 8610-RFP survives transiently in the cecum and colon after 4 d of oral administration of 8610-RFP to mice. (A) The experimental design. Mice were administered 8610-RFP for 4 d (5×10^9 CFU/d). Whole-body imaging was conducted (days 1–7) and euthanasia was performed daily with excision of the GITs for ex vivo imaging. Feces were collected daily from days 0–10. (B) The total fluorescence intensity [(p/s/cm^2/sr)/(μW/cm^2)] and its standard error for the whole animals of each group of five mice are shown. (C) A representative image of two mice, where the background is represented by (1) a negative control mouse and the experimental group is represented by (2) a mouse that ingested 8610-RFP for 4 d. (D) The mean and standard deviation (SD) of CFU counts for each group of five mice from days 0–10 are plotted. (E) Representative images of the 8610-RFP fluorescence found in the digestive tract of a mouse from days 0–7. The color bar indicates the radiant efficiency [(p/s/cm^2/sr)/(μW/cm^2)].

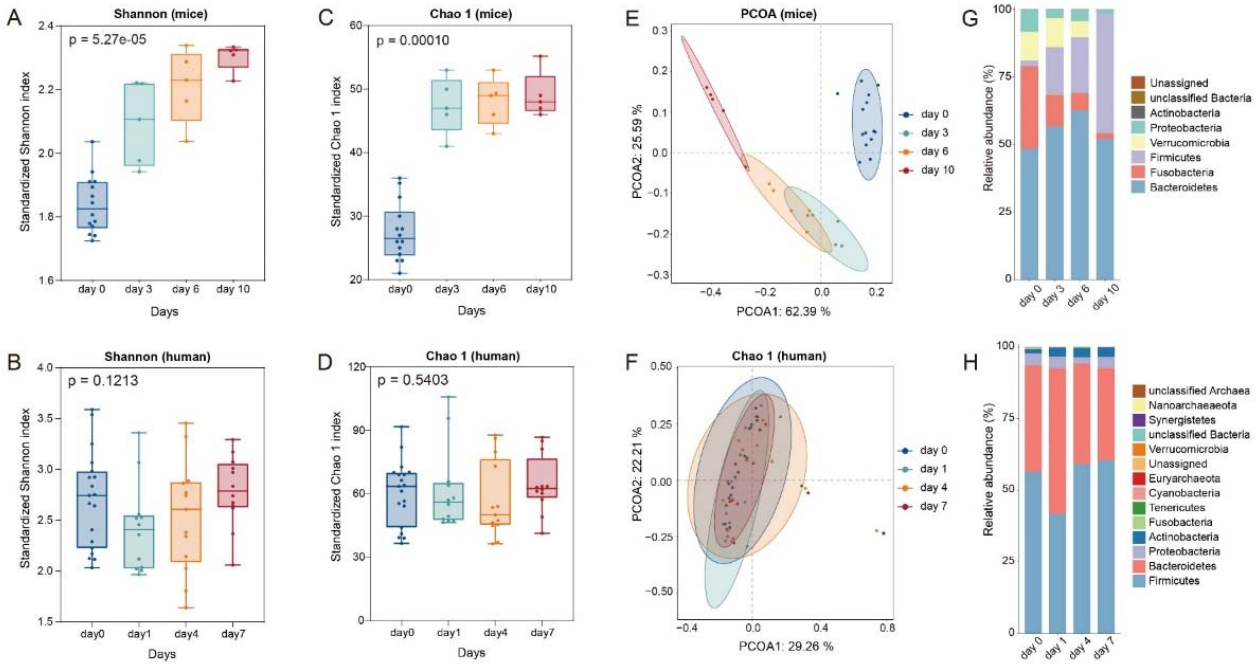
3.2 The ability of CCFM8610 to regulate the gut microbial community in humanized mice and clinical experimental corroboration

To confirm whether the short-term residence of *L. plantarum* affects the host, we further analyzed the gut microbial composition of humanized mice before and after 8610-RFP intake. The Shannon and Chao 1 indices of the intestinal microbiota of mice increased significantly after 8610-RFP ingestion, and this effect was somewhat persistent ($P < 0.05$; Figures 2A and C). PCOA analysis showed that the microbiota before 8610-RFP ingestion had a disparate microbial structure compared with that after ingestion, further indicating that CCFM8610 ingestion altered the gut microbial community of humanized mice (Figure 2E). The bacterial taxonomic profiles of the mice before and after the intervention were analyzed. The mouse microbiota consisted predominantly of Bacteroidetes, Fusobacteria, and Firmicutes before and after the intervention at the phylum level (Figure 2G). Samples after 8610-RFP gavage showed an expansion of Firmicutes but a decrease in the proportion of Fusobacteria, and these changes were somewhat persistent (Supplementary Figure 1A).

By comparing the results of the gut microbial changes in humanized mice with those of clinical trials, we showed that they were not entirely consistent. The Shannon and Chao 1 indices of the microbiota in participants' fecal samples were not significantly different (Figures 2B, D, and F), suggesting that short-term ingestion of CCFM8610 did not macroscopically alter the intestinal microbiota of the host. Consistently, at the

phylum level, the relative abundance of Fusobacteria decreased after intervention (Figure 2H, Supplementary Figure 1B).

To further analyze the persistence of the influence of CCFM8610 on the host gut microbiota, cluster analysis was performed on all genera according to changes in the relative abundance of different bacteria over time (Figure 2I). The relative abundances of clusters 2, 3, 4, and 5 increased significantly after 8610-RFP ingestion, and the bacterial abundances of clusters 2 and 5 continued to decrease after the cessation of the intervention. The abundance of Cluster 3 decreased slightly after cessation of the intervention and then increased on day 10. In contrast, the abundance of Cluster 4 peaked on day 6 and declined significantly by day 10. Notably, the relative abundance of bacteria in cluster 6 decreased after 8610-RFP ingestion and continued until day 10. Cluster analyses of human samples showed that the families exemplified by Veillonellaceae (Cluster g) showed changes similar to those of Cluster 2 in the mouse samples (Supplementary Figure 1C). Consistent with Cluster 6, bacteria belonging to the families Fusobacteriaceae, Enterobacteriaceae, and Prevotellaceae (Cluster f) declined after CCFM8610 ingestion. Clusters 7 and c (including Barnesiellaceae and Christensenellaceae) also showed similarities.



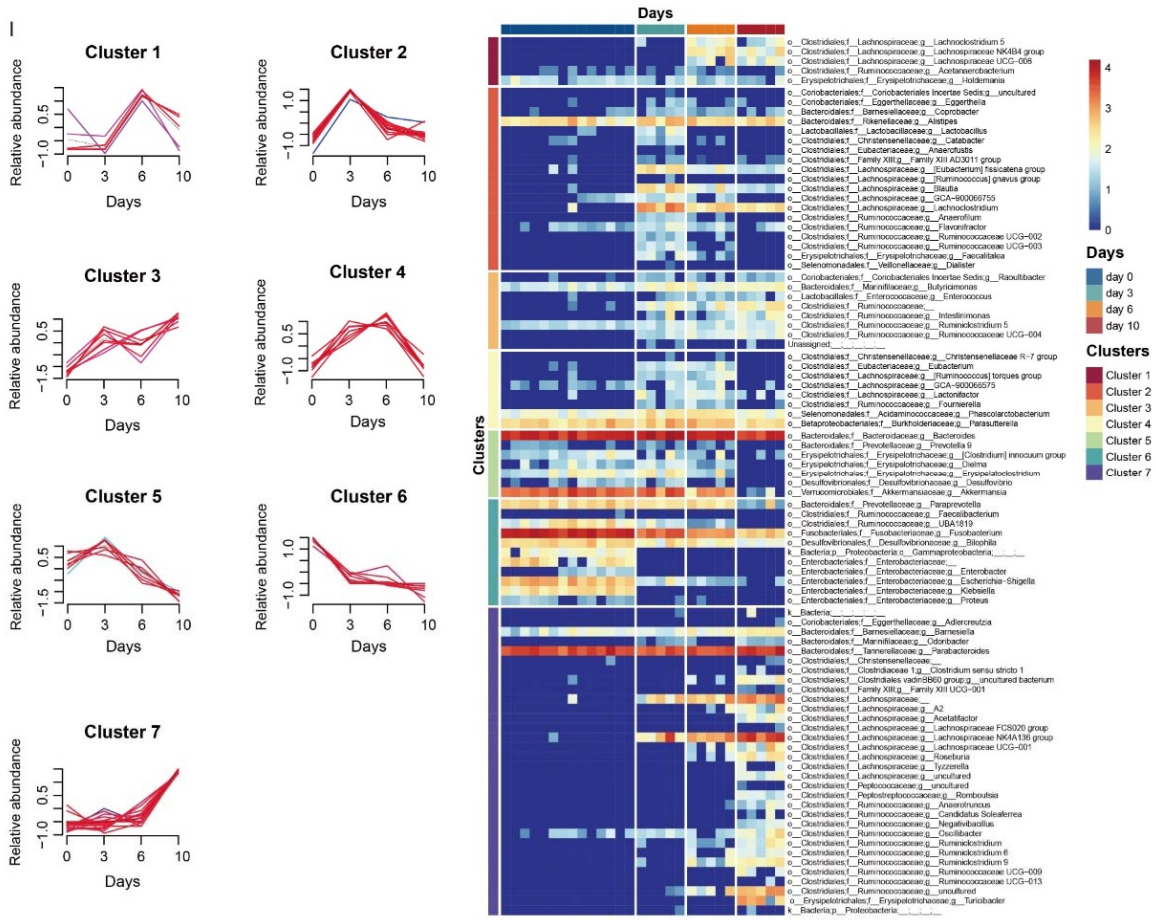


Figure 2. Comparison of microbial alterations in mice and humans before and after receiving 8610-RFP treatment. (A and B) Differences in the Shannon diversity index in mouse (A) and human (B) fecal samples on different days, as shown using the Kruskal Wallis test. (C and D) Differences in the Chao 1 diversity index in mouse (A) and human (B) fecal samples on different days, as shown using the Kruskal Wallis test. (E and F) PCoA of fecal samples from mice (E) and humans (F) on different days, plotted using the Bray-Curtis distance. Each point represents a sample. (G and H) Mean relative abundance of major phyla of fecal samples from mice (G) and humans (H) on different days. (I) Results of the Mfuzz clustering of genus levels of fecal samples from mice based on patterns of relative abundance over time and heatmap of genus-level abundance in seven clusters at multiple time points. Color bar ranging from blue to red represents relative abundance from low to high after normalization.

3.3 Alterations in microbial function after CCFM8610 intervention

After showing that CCFM8610 intake alters the abundance of specific gut microbiota with a sustained effect over time, we examined the alteration in function that may result from changes in microbial composition. To this end, we further explored the functional alterations at KO genes and pathway levels of KEGG. We identified 3505 differential KO genes in mouse samples and 2207 differential KO genes in human samples, respectively, of which 1137 differential KO genes were detected in both types of samples (Figure 3A).

At the metabolic pathway level, we identified 30 differential metabolic pathways in mouse samples and 85 differential metabolic pathways in human samples (Figure 3B). Of these, only five metabolic types, represented by lipid metabolism, showed significant variations in the mouse samples, whereas differential metabolic pathways were present in almost every category of metabolic type in the human clinical samples ($P < 0.05$; Figure 3B).

Of all the differential metabolic pathways, six were altered in both sample types (Figure 3C). Pathways involved in lipid metabolism were altered in the samples after CCFM8610 ingestion, such as steroid biosynthesis declining after a brief increase and secondary bile acid metabolism increasing. In addition, aminobenzoate degradation and lipopolysaccharide biosynthesis changed in both sample types; however, the trends were not entirely consistent. These results demonstrate that changes in microbial composition resulting from the intervention of CCFM8610 may lead to changes in microbial function, particularly in lipid metabolism, which are consistent across samples, and that these changes in function are likely to be reflected in the derived metabolites.

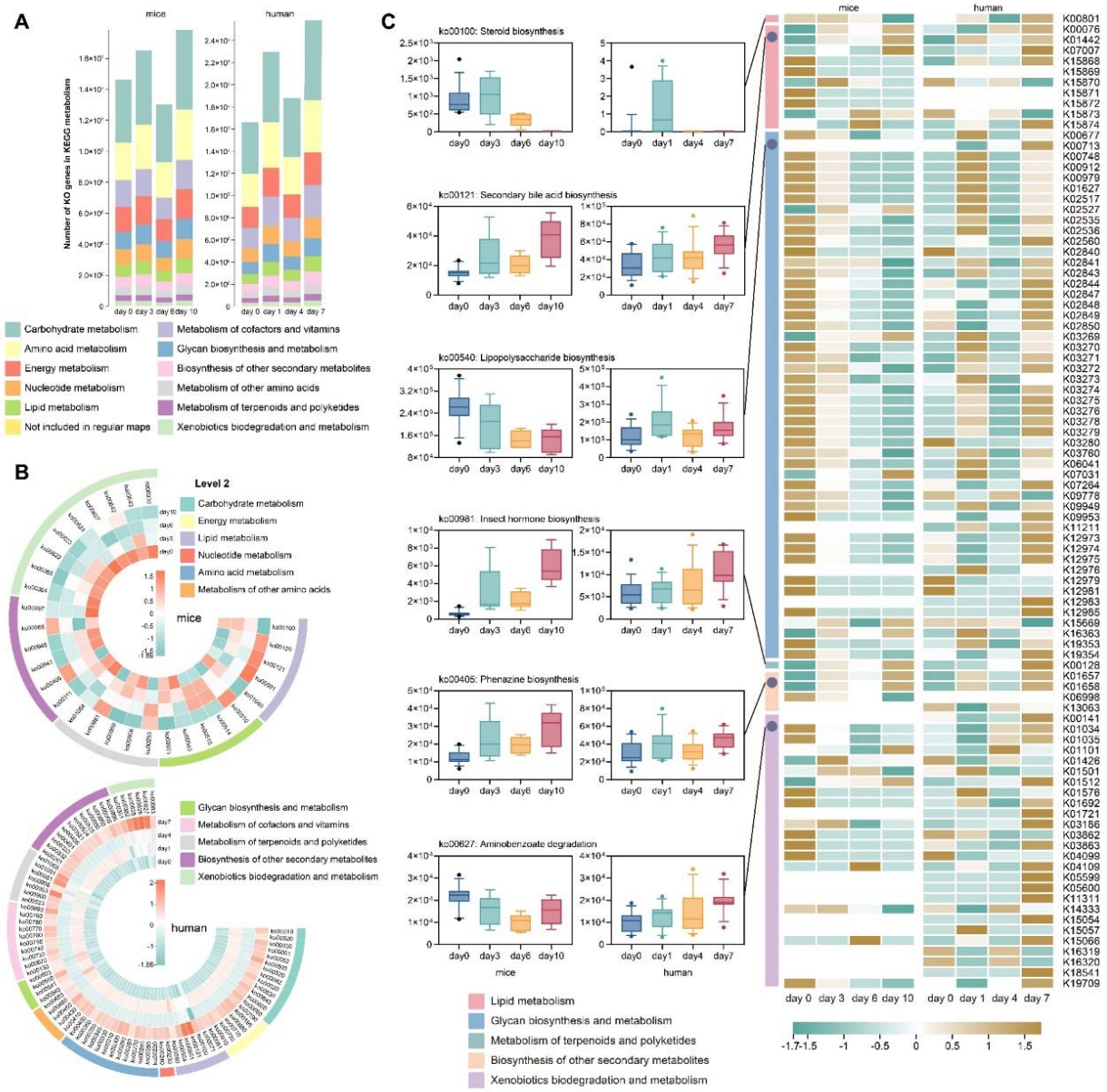


Figure 3. Alterations in microbial function in the mice (left) and human (right) pathways before, during, and after treatment with CCFM8610. (A) Taxa bar plot showing the number of KO genes in the second-level categories under KEGG metabolism. (B) Heatmap showing differential pathways in mouse (up) and human (down) samples before, during, and after receiving CCFM8610. (C) The box plots (left) show the relative abundance of shared differential pathway (identified through the Kruskal Wallis test) of mice (left) and human (right) before (blue), during (green), and after (yellow and red) receiving CCFM8610. All box plots represent the 25–75th percentiles of the distribution; the whiskers extend up to the 10–90th percentiles of the distribution and others are represented as dots. The heatmap (right) shows all KO gene expressions included in each differential metabolic pathway. The cell colour and shading represent the abundance of KO genes after normalization.

3.4 Altered host metabolic profiles and correlation with microbial function after intervention

Having shown that CCFM8610 may have a certain and sustained effect on lipid metabolism in the gut microbiota, we next elucidated whether this effect was reflected in specific metabolite changes in the microbiota. For this purpose, we conducted an untargeted metabolomic analysis of fecal samples from mice that ingested 8610-RFP. Metabolomic analysis revealed 43 differential metabolites, of which 10 were upregulated during 8610-RFP intake and 15 were downregulated after 8610-RFP intake; the abundance of the remaining 18 differential metabolites appeared to be elevated after cessation of the intervention (Figure 4A). Consistently, the classification of differential metabolites revealed that 41.18% of the compounds were lipids and lipid-like molecules, and 23.53% were organoheterocyclic compounds (Figure 4B). Similarly, of the 60 differential metabolites revealed in the clinical trials samples, excluding metabolites not in the Human Metabolome Database (HMDB), 22.92% of the differential compounds belonged to lipids and lipid-like molecules and 16.67% were organoheterocyclic compounds. However, the most predominant metabolites in the human samples compared to the mouse samples were organic acids and derivatives (43.75%).

Comparison of mouse differential metabolites with human differential metabolites revealed that the common differential metabolites were cholic acid, daidzein, guanine, and jasmonic acid (Figure 4C). We then combined the results of the microbial function prediction with the metabolome results and found that the bile acids in the differential metabolites belonged to secondary bile acid metabolism (ko00121) intermediates. These results corroborate our previous findings that changes in microbiota genome function can lead to alterations in derived metabolites. Lipid metabolism, exemplified by bile acid metabolism, may be an important metabolic pathway affected by CCFM8610 intake.

Given that bile acid metabolism is one of the most impacted pathways in functional prediction and untargeted metabolomics studies, we performed targeted bile acid analysis. After four consecutive days of oral administration of CCFM8610, when CCFM8610 abundance reached its peak in the gut, total bile acid levels were significantly elevated compared to baseline ($P < 0.05$, Mann-Whitney test) (Figure 4D). To further explore the potential links between microbial composition and metabolite changes, we constructed a bacteria-metabolite correlation network based on Spearman correlation. A total of 427 significantly correlated bacteria-metabolite pairs were identified ($|r| > 0.6$, $P < 0.05$), of which 206 were positively correlated and 221 were negatively correlated. Bile acid levels were significantly associated with several key bacterial groups, including *Akkermansia*, *Bilophila*, *Roseburia*, and *Fusobacterium*. It is worth noting that *Akkermansia*, *Bilophila* and *Fusobacterium* were positively correlated with the abundance of CCFM8610 and were reported to be involved in the regulation of secondary bile acid metabolism or lipid metabolism, suggesting that CCFM8610 may indirectly affect host lipid metabolism by regulating these bacterial communities.

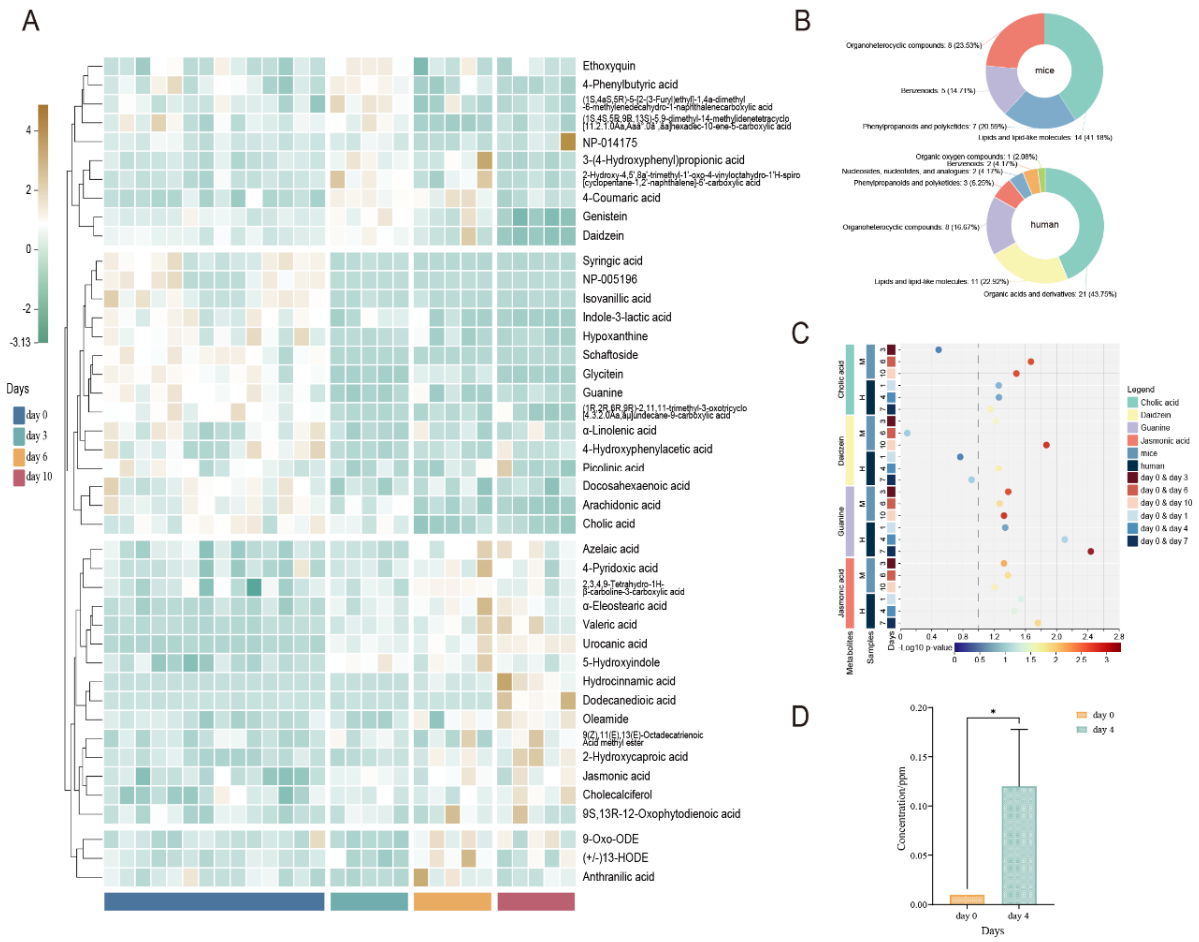


Figure 4. Metabolomics analyses revealed changes in intestinal metabolites in the samples after administration of CCFM8610. (A) Heat map analysis of differential metabolites in mouse fecal samples at different time points before and after receiving 8610-RFP. Each column in the heat map represents one sample. The cell color and shading represent the relative abundance of metabolites after normalization. (B) Classification of different metabolite compounds using the HMDB. Pie plot showing the classification in HMDB to which the differential metabolites belong and the relative proportion of metabolites in that classification. (C) Bubble plots illustrating co-differential metabolites in mouse and human samples. Each bubble represents the VIP value of the metabolite, indicating the contribution of the metabolite to the difference between two groups. The color gradient of bobble indicates the statistical significance (p value) of the metabolite between the two groups. The Wilcoxon test was used to calculate the p values. (D) Bar charts show the content and significance of bile acids in the intestine of mice on days 0 and 4 after CCFM8610 administration. P-values were calculated using the Wilcoxon test.

4. Discussion

The colonization of *L. plantarum* in the host intestine is crucial for its sustained beneficial effects. CCFM8610 is a typical *L. plantarum* strain with a wide range of beneficial effects, including alleviating intestinal diseases, modulating host immunity, and antagonizing acute cadmium toxicity in humans[18, 23, 24]. In this study, CCFM8610 was colonized in humanized mice and validated in clinical trials.

Humanized mouse colonization experiments showed that CCFM8610 has a short-term retention ability in the distal part of the host intestine, mainly distributed in the cecum and colon. Its fluorescence signal dropped rapidly after feeding was stopped, indicating that CCFM8610 lacks the ability to colonize the mouse intestine for a long time. This feature is consistent with previous studies, that is, most *Lactobacillus* spp. can exist in the intestine for a short period of time, but it is difficult to form stable colonization. The gastrointestinal persistence of *L. plantarum* WCFS1 in human volunteers can be detected within three days after the last intake, but it cannot be detected within seven days[25]. Catherine Daniel *et al.* [26]found that

fluorescently labeled *L. plantarum* only persisted in the intestine for a few days after the last dose; they were completely excreted with feces about a week after the last tube feeding, and did not colonize the intestine for a long time as previously observed.

Although CCFM8610 resides only briefly in the intestine, its ingestion significantly altered the gut microbiota structure of humanized mice. CCFM8610 promoted increased microbial diversity, elevated Shannon and Chao1 indices, and improved intestinal ecological stability. Community-level changes, such as an increase in Firmicutes and a decrease in Fusobacteria, further suggest that the gut microbiota may be undergoing a functional shift toward a more stable and beneficial microbial community structure. Members of Firmicutes play important roles in systemic immunity, including regulating myeloid cell lifespan, enhancing pulmonary antibacterial and antiviral immunity, and increasing resistance to sepsis[27], while Fusobacterium may be linked to colorectal cancer[28]. The bacterial community change trends we observed are consistent with those reported for other Lactobacillus strains. For example, Chin-Chu Chen *et al.* [29]found that at the family level, *L. plantarum* GKM3 significantly increased *Bacteroidaceae*, *Lactobacillaceae*, and *Verrucomicrobiaceae*, while decreasing *Alcaligenaceae* and *Fusobacteriaceae*. Zhenmin Liu et al. [30]demonstrated that *L. plantarum* ST-III reduced the total abundance of Enterobacteria and Bacteroidetes in the infant gut, while increasing the abundance of lactobacilli and certain *Bifidobacteria*. These results collectively support that different strains of *L. plantarum* can promote the maintenance of intestinal ecological balance and immune homeostasis by regulating the composition of the host intestinal flora.

Further comparison of clinical experimental results showed that there was no significant difference in the Shannon index and Chao1 index of the microbiota in human fecal samples, indicating that the basic flora status and host environment may affect the effect of probiotics. In addition, the probiotic effects of *Lactobacillus* spp. on healthy humans are more likely to be attributed to micro-regulation of the host's intestinal flora, rather than macro-changes in the diversity of the intestinal microbiome when the same amount of lactobacilli is ingested[31]. For example, after the ingestion of *L. plantarum* Lp082, the experiment did not significantly change the overall classification and functional composition of the intestinal flora at any time point, but had a strong effect on the structure and function of the mouse intestinal flora. This difference may be due to individual differences or higher resilience of the human intestinal flora[32]. However, consistent changes with those in mouse studies were still observed at the level of specific taxa, such as a decrease in the relative abundance of *Fusobacteriaceae* and *Enterobacteriaceae*.

In addition, we further demonstrated that CCFM8610 could still maintain its probiotic function for a period of time after cessation of intake. Our functional prediction results showed that CCFM8610 intervention caused functional changes in both mouse and human samples. CCFM8610 may cause the intestinal microbiota of healthy subjects to be more functional than before intake, among which lipid metabolism is the most prominent target. Previous studies have reported that the YS5 strain exhibits a high cholesterol removal ability and has a significant effect on lowering serum cholesterol[33]. Non-targeted metabolomics further confirmed the persistent effect of CCFM8610 on intestinal microbial metabolism, especially in lipid-related pathways.

Whether in mice or clinical samples, a considerable proportion of the differential metabolites belong to lipids and their analogs, which is highly consistent with the lipid metabolism changes obtained by functional prediction. It is worth noting that differential metabolites such as cholic acids and soybean flavonoids were detected in both mouse and human samples, suggesting that CCFM8610 may induce cross-species metabolic responses.

L. plantarum can induce fecal bile acid secretion, thereby promoting the decomposition of liver cholesterol into bile acid[34]. For example, *L. plantarum* KLDS 1.0344 can accelerate the conversion of liver cholesterol into bile acid by upregulating the expression of the CYP7A1 gene[35]. In this study, we found that mice ingesting CCFM8610 had elevated bile acid levels. However, another study has reported that *L. plantarum* LP06CC2 tends to increase total bile acid levels in the cecum while significantly reducing the proportion of deconjugated bile acids[36]. This result differs from our observations, possibly due to strain-specific differences, and LP06CC2 may alter the composition of bile acids by inhibiting intestinal reabsorption. The results of this study also suggest that CCFM8610 may regulate bile acid synthesis and circulation through similar mechanisms, thereby affecting the host's lipid metabolism balance. The role of bile acid in lipid metabolism regulation has long been reported: on the one hand, it acts as a surfactant to promote lipid digestion and absorption; on the other hand, it acts as a signaling molecule to regulate lipid synthesis, transport and oxidation in the liver and peripheral tissues through receptors such as FXR and TGR5[37]. Studies have shown that BSH1 activity significantly changes the expression of key regulators of lipid metabolism in the ileum and liver, and that bile acid dissociation in the gastrointestinal tract will cause significant local and systemic changes in lipid metabolism[38]. The reason why lipid metabolism has become the most prominent target may be that bile acid, as a key mediator of microbial-host interaction, plays a core hub role in the regulation of lipid homeostasis.

In summary, our study demonstrates that ingested CCFM8610 can transiently reside in the ceco-colonic region of the host gut. *Lactobacillus* spp. do not alter overall microbial homeostasis but do induce subtle adjustments in gut microbiota composition. Furthermore, our results show that CCFM8610 ingestion has consistent effects on lipid metabolism, particularly bile acid metabolism, in both mouse and human samples. Given the central role of bile acids as regulators of lipid absorption and metabolic signaling, these results highlight the potential of CCFM8610 as a probiotic candidate to modulate host lipid metabolism. Clinically, this may have implications for metabolic health management, such as improving cholesterol homeostasis or preventing dyslipidemia. However, it should be noted that our intervention was short-term, and the effects observed at the human population level were modest. Therefore, future clinical studies with larger sample sizes, longer intervention periods, and targeting host metabolic endpoints (e.g., serum lipid profiles, bile acid signaling pathways) are warranted to further evaluate the therapeutic potential of CCFM8610.

Code availability

All code used in analyses are available by request from the corresponding author.

Funding

This work was supported by the National Natural Science Foundation of China [No. 32425044 and U23A20259], and supported by the Fundamental Research Funds for the Central Universities JUSRP622013 and Collaborative innovation center of food safety and quality control in Jiangsu Province.

Ethics approval and consent to participate

The animal care and study protocols were approved by the Ethics Committee of Jiangnan University, China (JN.No20220315c1800630[058]). The human study was approved by the Medical Ethics Committees of Jiangnan University, and was registered in the Chinese Clinical Trial Registry (clinical trial number: JNU20230601IRB14).

Conflicts of interest

The authors declare no Competing Financial or Non-Financial Interests.

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