

Original Article



## Investigation of the effect of oligo-xylulose on the intestinal flora under pressure overload in mice based on 16S rRNA gene sequencing

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### Article Info

### Abstract



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The relationship between gut microbiota dysbiosis and heart failure has been drawing increasing attention. This study aimed to investigate the effects of oligo-xylulose (XOS) on the gut microbiota of mice with heart failure induced by pressure overload. A chronic heart failure mouse model was constructed by pressure overload, and XOS was administered in their diet. The gut microbiota was analyzed using 16S rRNA gene sequencing, and the effects of XOS on the microbiota composition were evaluated. XOS supplementation improved the balance of intestinal microbiota in mice under pressure overload, increasing the abundance of beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus*, while decreasing the abundance of harmful bacteria, such as *Desulfovibrio* and *Enterococcus*. XOS has the potential as a dietary supplement to improve the balance of intestinal microbiota and benefit individuals with heart failure. The findings of this study suggest that modulating the gut microbiota could be a novel strategy for treating heart failure.

**Keywords:** Oligo-xylulose, Gut microbiota, Heart failure, Pressure overload, 16S rRNA gene sequencing

## 1. Introduction

Heart failure is an abnormal change in the structure and/or function of the heart due to various reasons, which causes obstacles in ventricular contraction and/or relaxation, leading to a complex set of clinical syndromes [1,2]. The human gastrointestinal microbiota is a complex microecosystem that affects various physiological functions of the intestines and plays a vital role in the body's nutritional regulation, maintenance of the integrity of the intestinal epithelial barrier, and development of mucosal immunity [3,4]. In chronic heart failure, when the intestines become ischemic and their permeability increases, the balance of the intestinal microecology is disrupted, leading to dysbiosis. This results in the release of various toxic substances, which in turn exacerbate heart failure [5,6].

Xylo-oligosaccharides (XOS) are a type of oligosaccharide with dietary fiber function, which has been proven to improve the balance of intestinal microbiota and benefit individuals with metabolic abnormalities [7,8]. XOS consists of xylo-oligosaccharides linked by  $\beta$ -(1-4) bonds, which resist digestion in the human small intestine [9]. XOS selectively promotes the growth of *bifidobacteria* and *lactobacilli* and inhibits conditionally pathogenic bacteria. XOS offers a high efficacy-to-cost ratio, requiring only a

small dose to achieve its desired effect in regulating the gut microbiota, with fewer intestinal side effects, making it convenient for clinical application [10]. Research has found that doses of XOS ranging from 1.2 to 8g per day effectively stimulate the growth of bifidobacteria [11]. The selective proliferation effect of XOS on beneficial bacteria optimizes the gut microbiota, improving the balance and function of the gut microecology [12,13].

At the same time, a low dose of XOS dietary supplements can improve blood sugar regulation, reduce pro-inflammatory cytokines, and increase the relative proportion of fecal *bifidobacteria* [14]. XOS can also improve gastrointestinal health by producing short-chain fatty acids (SCFAs) through the fermentation of *bifidobacteria* and reducing colonic pH, thereby inhibiting the proliferation of pathogenic and inherent putrefactive bacteria [13]. Among SCFAs, butyric acid can reduce the expression of invasion genes of harmful bacteria and decrease the attack of Salmonella on epithelial cells, exhibiting a strong killing effect on Salmonella. XOS itself also has antibacterial properties. Purified acidic XOS has antibacterial activity, inhibiting the growth of both Gram-negative and Gram-positive aerobic bacteria and *Helicobacter pylori*. XOS can competitively bind to lectins on the surface of pathogens,

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reducing the chance of pathogens binding to intestinal mucosal epithelial cells [15]. It can even displace the glycosyl part of intestinal mucosal cells that have already bound to lectins, depriving the pathogens of their virulence. Recent studies have found that under the intervention of XOS supplements, kidney function in mice with chronic kidney disease can be improved by affecting microbial composition and metabolic changes. In addition, XOS can reduce obesity by lowering the gene expression of fat formation and synthesis markers and can induce changes in the composition of gut microbiota [16].

In summary, heart failure can lead to intestinal ischemia, damage to the mucosal barrier, changes in the intestinal immune environment, and dysbiosis. The effects of XOS on regulating intestinal flora, reducing inflammatory responses, and its role in fat metabolism have been supported by many studies. However, it is still unclear whether XOS can regulate the intestinal flora under stress load conditions and whether this function can improve heart function or reverse myocardial remodeling. In this study, we hypothesize that by establishing a pressure-loaded chronic heart failure mouse model. We can utilize the benefits of XOS in regulating gut flora, and its antibacterial and immunomodulatory effects, to observe its potential improvement in the development of heart failure in mice. We will study its mechanism of action from the perspective of regulating gut flora.

## 2. Materials and Methods

### 2.1. Establishment of the TAC Chronic Heart Failure Model in Mice

Mice of the C57BL/6J strain, aged 5-7 weeks, were divided into three groups based on a similar weight distribution principle. Experimental groups were designated as follows: Sham surgery group (Sham), model group (TCA), and treatment group (TCAY) (17,18). After being fed a standardized mouse-specific diet for 3 weeks, they underwent TAC surgery (details below). In the 8 weeks following surgery, Group A (20 mice) continued on the initial diet, Group B (20 mice) received a diet supplemented with XOS (2%), and Group C (20 mice) received a diet supplemented with XOS (7%). In each group, three mice underwent sham surgery (chest opened without aortic constriction) serving as surgical blank controls.

### 2.2. 16S rRNA Analysis of Intestinal Microbiota

The heating block was pre-warmed to 85°C. A denaturation system of 10 µL was prepared: 1000 ng of RNA, 2 µL of 5× loading buffer, and DEPC water topped up to volume (all operations were performed on ice) in a 600 µL Eppendorf tube. After instant separation of the denaturation system, it was denatured in the heating block for 10 minutes and then cooled on ice for another 10 minutes. 1 µL of 0.1% EB was added to the tissue RNA denaturation system, mixed thoroughly, and then loaded into the gel wells. The Roche RNA reverse transcription kit (100 reactions) was used, with reactions conducted in a Gene Amp 2400 PCR machine. cDNA quantification was achieved using the NanoDrop 2000 UV-Vis Spectrophotometer.

Bacterial universal primers 27-F-AGAGTTTGATCC-TGGCTCAG and 1492-R-GGTTACCTTGTTACGACTT were selected for amplification of the full bacterial 16S rRNA gene. Following the specific operational instructions, an overview is as follows: PCR products of the

intestinal microbiota from each group of mice were individually ligated to vectors and transformed into competent *E. coli* cells. Positive clones were selected, recombinant plasmids were extracted, and a 16S rRNA gene library was constructed. The positive clone plasmid DNA underwent PCR amplification for 16S rRNA fragments, followed by a restriction enzyme digestion test. Accordingly, the types and numbers of intestinal microbiota corresponding to each group of mice were statistically analyzed.

### 2.3. OTUs Analysis

Raw sequencing data were assembled and filtered to obtain valid data. Based on this valid data, Operational Taxonomic Units (OTUs) clustering, species annotation, and abundance analysis were carried out. T-tests were then performed to identify differences in community structure between samples. The 16S rDNA gut microbiota structure spectrum sequencing analysis was conducted on the Illumina HiSeq sequencing platform. A short fragment library was constructed using paired-end sequencing. By filtering and assembling the raw sequencing sequences, optimized sequences (Tags) were obtained. These optimized sequences were then clustered to define OTUs, and the species classification was determined based on the sequence composition of the OTUs.

### 2.4. Alpha and Beta Diversity of Mouse Intestinal Microbiota

Based on the OTU analysis results, further analyses were performed, including Alpha diversity, Beta diversity, and significant species difference analyses, to explore differences between samples. A rarefaction curve, one of the analyses for both Alpha and Beta diversity, measures whether the sample data volume is sufficient to reflect the species diversity within the sample. The QIIME software was used for Beta diversity analysis, which compares the degree of similarity in species diversity among different samples. ANOSIM analysis, a type of Beta diversity analysis, calculates the distance of Beta diversity between samples using four different algorithms. Unweighted unifrac and Jaccard are non-weighted algorithms, while Weighted unifrac and Braycurtis are weighted algorithms. Non-weighted algorithms reflect species presence or absence, while weighted algorithms consider both species presence or absence and their abundance. The RDP classifier Bayesian algorithm was used to taxonomically analyze the representative sequences of OTUs at a 97% similarity level.

### 2.5. Lefse (Linear Discriminant Analysis Effect Size)

Analysis Lefse (Linear Discriminant Analysis Effect Size) analysis was employed to identify marker species with statistical differences between groups. In this experiment, the filtering criterion was set to an LDA score > 4. Metastats analysis used the Metastats software to perform a t-test on species abundance data between groups. KEGG analysis was completed using the PICRUSt software. The primers used for amplification targeted the bacterial 16S rDNA (V3+ V4) region: 338F: 5'-ACTCCTACGG-GAGGCAGCA-3', 806R: 5'-GGACTACHVGGGTWCT-TAAT-3'. DNA extraction, PCR amplification, and sequencing were collaboratively carried out by Biomarker Technologies Co. Ltd.

## 2.6. Tax4Fun Functional Prediction

High-throughput 16S sequencing data, processed through the QIIME or SILVAngs platform, were taxonomically classified for OTUs based on the SILVA database. Next, 16S copy numbers were normalized according to the genome annotations from NCBI. Lastly, by constructing a linear relationship between the SILVA classification and prokaryotic classification in the KEGG database, a prediction of microbial gene functions was realized. In this study, functional distribution charts were generated based on the top 20 predictions by abundance.

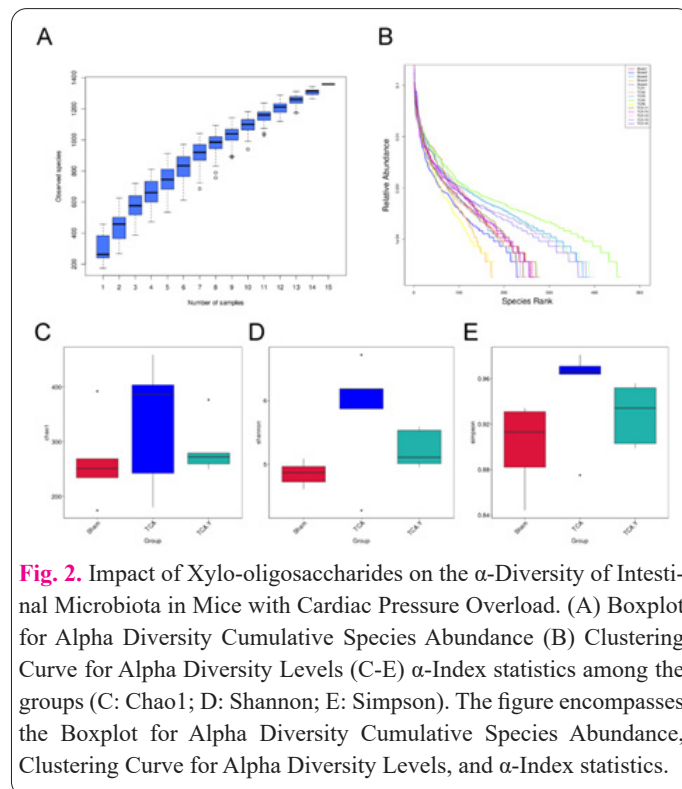
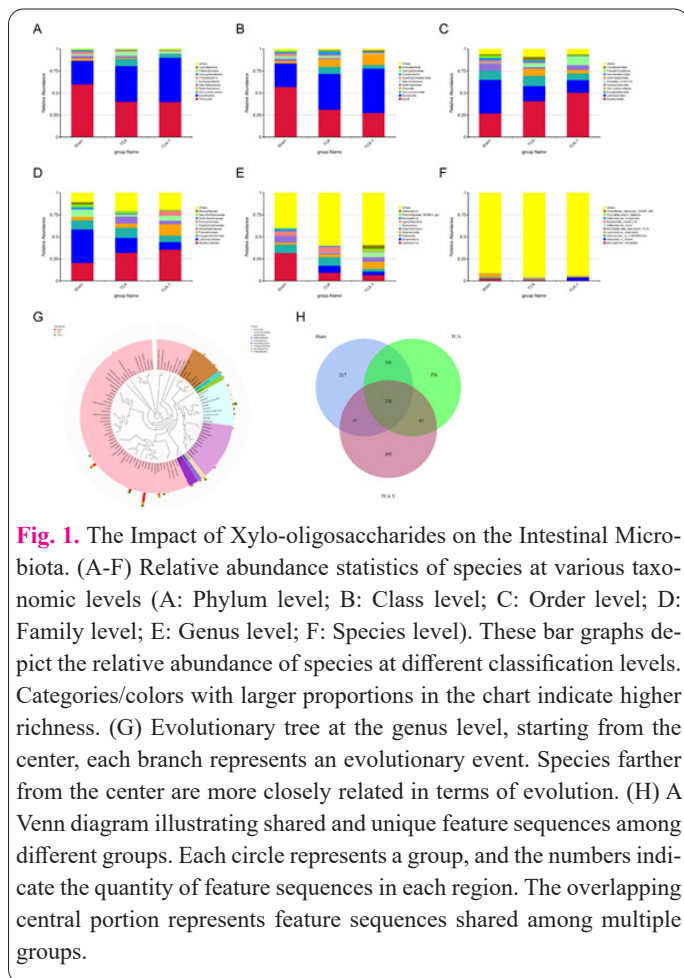
## 2.7. Statistical analysis

Each experimental data set was represented by the average  $\pm$  standard deviation. Comparisons between multiple groups were made using one-way ANOVA with the least significant difference (LSD) method, with  $P < 0.05$  considered statistically significant.

## 3. Results

### 3.1. The Impact of Xylo-oligosaccharides on the Intestinal Microbiota

Composition in Mice with Cardiac Pressure Overload We first investigated the effect of xylo-oligosaccharides on the composition of intestinal microbiota in mice with cardiac pressure overload. The results, depicted in Figures 1A-G, show that at the genus level, there were significant changes in microbial abundance among the groups. The dominant bacteria in the Sham and TCA groups were *Lactobacillus* and *Dubosiella*, while in the TCA.Y group, the dominant bacteria were *Lactobacillus* and *Alloprevotella*, with *Alloprevotella* showing the most significant relative abundance change. There were also noticeable changes



in species abundance at other taxonomic levels among the groups. Figure 1H illustrates that the characteristic sequences of the three groups were quite distinct. While they each had their unique characteristic sequences, some sequences were shared among them. This indicates that while there were commonalities in the intestinal microbiota composition among the groups, each group also exhibited its distinct features. These results suggest potential changes in the species compositional structure within each group.

### 3.2. Impact of Xylo-oligosaccharides on the $\alpha$ -Diversity of Intestinal Microbiota in Mice with Cardiac Pressure Overload

We subsequently analyzed the  $\alpha$ -diversity of the microbiota. As shown in Figure 2A, the boxplot trends towards stability, indicating that the sampling was sufficient and data analysis could proceed. Figure 2B: The rarefaction curves for each group eventually level off, suggesting that the sequencing data is progressively reasonable and that an increase in data volume would no longer affect the alpha index. Figures 2C-H: The Chao1, Simpson, and Shannon indices for the Sham and TCA.Y groups were lower than those for the TCA group. No significant differences were observed between the Sham and TCA.Y groups. These results indicate that the microbial community complexity in the TCA group is higher compared to both the Sham and TCA.Y groups.

### 3.3. Impact of Xylo-oligosaccharides on the $\beta$ -Diversity of Intestinal Microbiota in Mice with Cardiac Pressure Overload

Further analysis of the microbiota's  $\beta$ -diversity revealed the following: As shown in Figure 3A, cluster analysis indicates that the Sham and TCA.Y groups are the most closely related. Figures 3B-C demonstrate that the Weighted Unifrac distances of the Sham and TCA.Y groups are significantly larger than those of the TCA group. These

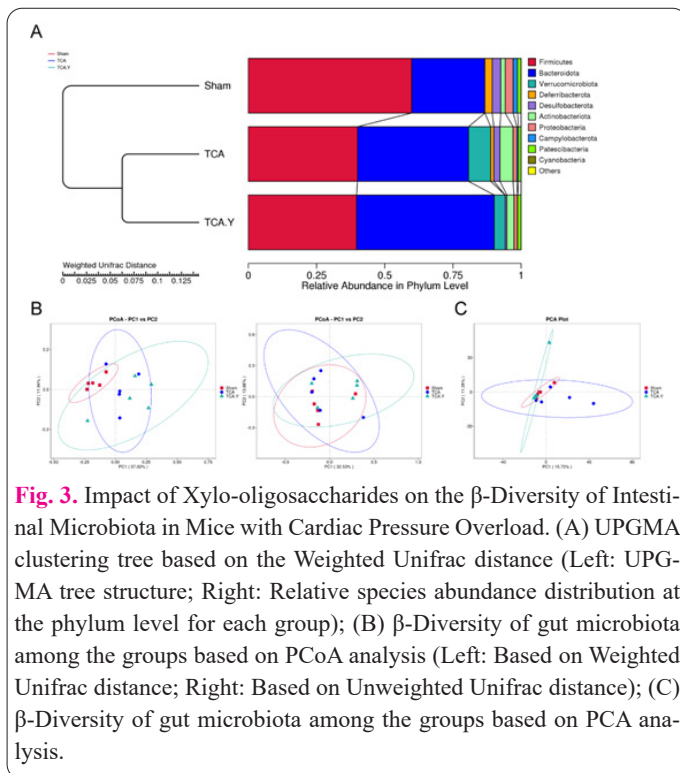
**Table 1.** Verification of inter-group community differences based on ANOSIM.

Group	R-value	P-value
Sham-TCA	0.716	0.005
Sham-TCA.Y	0.648	0.009
TCA-TCA.Y	0.54	0.007

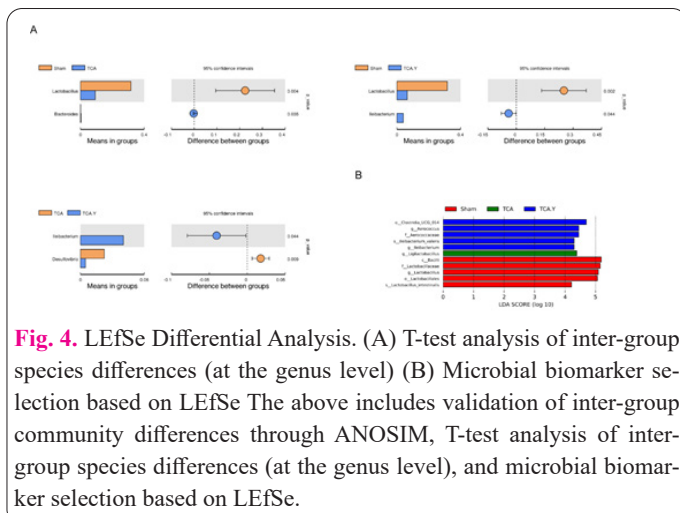
results suggest that there are certain differences in species composition structure among the groups. As can be seen in Table 1 above, the inter-group differences exceeded the intra-group differences, indicating that the grouping is meaningful.

### 3.4. LefSe Differential Analysis

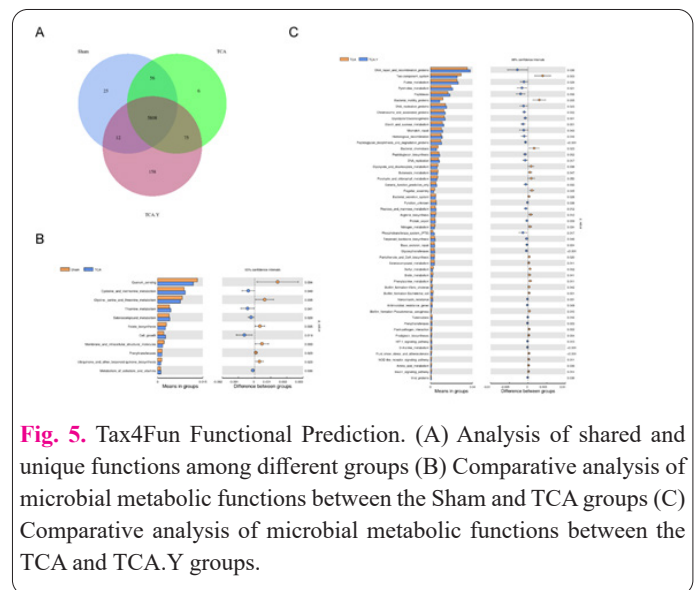
Upon further analysis of specific bacterial species, as shown in Figure 4A, when comparing the TCA group to the Sham group, *Lactobacillus* was significantly downregulated while *Bacteroides* was significantly upregulated. Comparing the Sham group to the TCA.Y group, *Lactobacillus* was markedly downregulated, and *lleibacterium* was notably upregulated. When comparing the TCA group to the TCA.Y group, *Desulfovibrio* was significantly upre-



**Fig. 3.** Impact of Xylo-oligosaccharides on the  $\beta$ -Diversity of Intestinal Microbiota in Mice with Cardiac Pressure Overload. (A) UPGMA clustering tree based on the Weighted Unifrac distance (Left: UPGMA tree structure; Right: Relative species abundance distribution at the phylum level for each group); (B)  $\beta$ -Diversity of gut microbiota among the groups based on PCoA analysis (Left: Based on Weighted Unifrac distance; Right: Based on Unweighted Unifrac distance); (C)  $\beta$ -Diversity of gut microbiota among the groups based on PCA analysis.



**Fig. 4.** LefSe Differential Analysis. (A) T-test analysis of inter-group species differences (at the genus level) (B) Microbial biomarker selection based on LefSe The above includes validation of inter-group community differences through ANOSIM, T-test analysis of inter-group species differences (at the genus level), and microbial biomarker selection based on LefSe.



**Fig. 5.** Tax4Fun Functional Prediction. (A) Analysis of shared and unique functions among different groups (B) Comparative analysis of microbial metabolic functions between the Sham and TCA groups (C) Comparative analysis of microbial metabolic functions between the TCA and TCA.Y groups.

gulated, while *lleibacterium* was considerably downregulated. Figure 4B indicates that at the genus level, *lleibacterium* serves as a biomarker for the TCA.Y group. At the species level, the *Valens* species within the *lleibacterium* genus is a biomarker for the TCA.Y group. These results suggest that *lleibacterium* might play a pivotal role in drug therapy.

### 3.5. Tax4Fun Functional Prediction

Lastly, through the Tax4Fun functional prediction analysis, as depicted in Figure 5A, there are evident variations in the distribution of gene numbers across the three groups, with both unique and shared genes present. Figure 5B demonstrates that, compared to the Sham group, pathways like Quorum sensing, glycine, serine and threonine metabolism, and Cell growth in the TCA group were significantly downregulated. Conversely, pathways such as Cysteine and methionine metabolism, and Thiamine metabolism were notably upregulated. Figure 5C indicates that, compared to the TCA group, pathways like DNA repair and recombination proteins, Purine metabolism, and Pyrimidine metabolism in the TCA.Y group were significantly upregulated, while the Two-component system and Bacterial motility proteins were markedly downregulated. These results suggest that the metabolic pathways encoded by the microbial communities in each group vary, indicating differing metabolic processes encoded by the microbiota. The microbiota exert regulatory effects through modulating related metabolic pathways.

## 4. Discussion

Heart failure is a complex clinical syndrome resulting from abnormal changes in the structure and/or function of the heart, leading to disorders in ventricular contraction and/or relaxation [19,20]. The human gut microbiota is a multifaceted microecosystem that influences a myriad of gastrointestinal physiological functions. During chronic

heart failure, the intestine becomes ischemic, its permeability increases and the balance of the gut microecology is disrupted [21,22]. This can lead to dysbiosis of the gut microbiota, releasing various toxic products and inflammatory factors, which in turn exacerbate heart failure [22]. This study primarily investigates the effects of xylo-oligosaccharides on the gut microbiota in a mouse model of cardiac failure. Using 16S rRNA gene sequencing technology, we explored the impact of XOS on the gut microbiota of mice. Experimental results demonstrate that XOS can significantly improve mouse cardiac function and regulate the gut microbiota towards a healthier state. The regulatory effect of XOS on the gut microbiota may be associated with its beneficial impact on cardiac function [23,24]. Additionally, this research delves into the regulatory mechanisms of XOS on the gut microbiota, encompassing its proliferation of beneficial bacteria and inhibitory effects on harmful bacteria. Heart failure patients exhibit a decline in gut microbiota diversity and alterations in its composition, which might be related to the onset and progression of heart failure. xylo-oligosaccharides have the capability to regulate the gut microbiota, potentially ameliorating the balance of the gut microecology. Figures 1-4 depict the influence of XOC on the gut microbiota composition in mice subjected to cardiac stress. Specifically, XOC can significantly affect the species composition and abundance of the mouse gut microbiota, likely due to its regulatory effects on the gut microbiota. Furthermore, XOC can enhance the diversity of the gut microbiota, potentially aiding in improving the gut microecological balance, thereby having a positive impact on the onset and progression of heart failure [24]. Heart failure can lead to intestinal ischemia, damage to the mucosal barrier, and alterations in the intestinal immune environment, resulting in gut microbiota dysbiosis. Conversely, the imbalance in the gut microbiota, bacterial translocation, and the release of harmful metabolic products, such as endotoxins, can stimulate inflammatory responses, damage cardiac cells, and further intensify the development and deterioration of heart failure.

XOS has a pronounced proliferative effect on *Bifidobacteria* and *Lactobacilli* while inhibiting potentially pathogenic bacteria. In the context of mice with cardiac load, XOS has exerted significant influences on the composition of the intestinal microbial species [13]. Compared to the control group, beneficial bacteria in the intestines of the XOS-treated mice increased in abundance, while some potentially harmful bacteria decreased. This suggests that XOS might exert its protective effects against heart failure by modulating the structure of the gut microbiota. XOS significantly increased the alpha diversity of the gut microbiota in mice under cardiac stress, implying that XOS might help maintain the stability of the gut microbiota, offering protection against heart failure. There were significant differences in beta diversity of the gut microbiota between the XOS-treated group and the control group, further confirming the modulatory role of XOS on the gut microbiota structure [25]. In this study, LEfSe analysis revealed the bacteria species with significant differences between the XOS-treated group and the control group, providing insights into how XOS might counteract heart failure by modulating the gut microbiota.

Drawing upon previous research, the relationship between gut microbiota and heart failure has garnered

extensive attention. Dysbiosis of the gut microbiota might be associated with the onset and progression of heart failure. Predictions from Tax4Fun can anticipate the metabolic pathways and functionalities of the gut microbiota. Therefore, there's a close connection between the gut microbiota and the differential pathways in Tax4Fun predictions. In general, the relationship between gut microbiota and differential pathways in Tax4Fun predictions, and the connection between these differential pathways and heart failure, constitute a complex topic that warrants further exploration. By integrating the relationship between the gut microbiota and the differential pathways in Tax4Fun predictions and the association between these pathways and heart failure, we can infer that the metabolic functionalities of the gut microbiota are closely related to the onset and progression of heart failure. Previous studies support this conclusion. For instance, one study found that the metabolic functionalities of the gut microbiota in heart failure patients differed significantly from those in healthy individuals [26]. These differences were closely linked to the onset and progression of heart failure. Another study discovered that modulating the metabolic functionalities of the gut microbiota could ameliorate the symptoms and cardiac functions of heart failure patients [14]. Thus, our research findings resonate with those of previous studies, suggesting that modulating the metabolic functionalities of the gut microbiota might emerge as a novel strategy for treating heart failure [27,28]. Our results indicate that, compared to the sham group, certain metabolic pathways were notably downregulated in the TCA group, while others were upregulated. This could suggest that the microbiota in the TCA group might be inhibited in some metabolic processes and promoted in others. These outcomes indicate that different microbial combinations might lead to different metabolic functionalities, aligning with previous research asserting that the composition and functionalities of the gut microbiota are intimately linked with the host's health status. For example, certain beneficial microbiota might exert favorable effects on the host through the production of metabolic products like short-chain fatty acids, while certain harmful microbiota might have adverse effects through the release of toxins or pro-inflammatory factors [29]. Concerning the relationship between gut microbiota and heart failure, prior studies have indicated that dysbiosis of the gut microbiota might be associated with the onset and progression of heart failure [30]. For instance, certain microbiota might impact the host's cardiovascular system through the production of specific metabolic products, leading to the onset of heart failure. Moreover, the gut microbiota might also affect cardiovascular health by influencing the host's immune system and inflammatory responses [30,31].

This research, by constructing a chronic heart failure mouse model and intervening with xylo-oligosaccharides, probes the effects of XOS on the gut microbiota and its underlying mechanisms. The relationship between gut microecological balance and heart failure has been drawing increasing attention. Our study offers a fresh perspective and approach to exploring the relationship between heart failure and gut microbiota dysbiosis. As a potential therapeutic agent, the regulatory effects of XOS on the gut microbiota could pave the way for new strategies in treating heart failure.

## 5. Conclusions

This study delves into the effects of xylo-oligosaccharides on the gut microbiota of mice with heart failure. By constructing a chronic heart failure mouse model induced by pressure overload and administering xylo-oligosaccharides in their diet, we observed its impact on the progression of heart failure in mice. The research further investigated its mechanism of action, focusing on the regulation of the gut microbiota. The findings unearth the potential link between gut microbiota and heart failure, introducing a novel strategy for treating heart failure by modulating the gut microbiota. This offers a fresh perspective and approach to heart failure treatment, aiming to enhance the quality of life and prognosis for patients.

## Conflict of Interests

The author has no conflicts with any step of the article preparation.

## Consent for publications

The author read and approved the final manuscript for publication.

## Ethics approval and consent to participate

This study was approved by the Animal Ethics Committee of the 2nd Xiangya Hospital of Central South University Animal Center.

## Informed Consent

The authors declare not used any patients in this research.

## Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Authors' contributions

Jianfeng Long and Kang Zhou designed the study and performed the experiments, Xiaoqi Qi collected the data, Zhenjie Tang analyzed the data, Jianfeng Long and Kang Zhou prepared the manuscript. All authors read and approved the final manuscript.

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