Heliyon 9 (2023) e16166

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Intestinal mucosal flora of the intestine-kidney remediation process of diarrhea with deficiency kidney-yang syndrome in Sishen Pill treatment: Association with interactions between *Lactobacillus johnsonii*, Ca^{2+}-Mg^{2+}-ATP-ase, and Na^{+}-K^{+}-ATP-ase

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ARTICLE INFO

Keywords:
Sishen Pill
Diarrhea with kidney-yang deficiency syndrome
Intestinal mucosal flora
Kidney
Energy metabolism

ABSTRACT

This study aims to investigate the effect of Sishen Pill on the characteristics of gut mucosal microbiota in diarrhea mice with deficiency kidney-yang syndrome. Fifteen Kunming male mice were randomly divided into Normal control group (C), Model self-healing group (X) and Sishen Pill group (S), with 5 mice/cages. Hematoxylin eosin (HE) staining was used to observe the kidney structure. Serum Na^{+}-K^{+}-ATP-ase and Ca^{2+}-Mg^{2+}-ATP-ase were detected by enzyme-linked immunosorbent assay (ELISA), Analysis of intestinal mucosal flora using third-generation high-throughput sequencing. The relative abundance results in the three groups revealed that the dominant bacterial genera: *Lactobacillus*, *Muribaculum* and *Candidatus-Arthromitus*; bacterial species: *Lactobacillus johnsonii*, *Lactobacillus reuteri*, *Lactobacillus murinus*, and *Lactobacillus intestinalis*, and differences in the presence of major microbiota between the X and S groups. A positive correlation between *Lactobacillus johnsonii* and both Ca^{2+}-Mg^{2+}-ATP-ase and Na^{+}-K^{+}-ATP-ase was found via correlation analysis. Sishen Pill also changed the manufacture of other secondary metabolites, as well as the metabolism of carbohydrates, glycans, energy, lipids, and other amino acids, and xenobiotics biodegradation and metabolism. In conclusion, Sishen Pill improved kidney structure, energy metabolism and the diversity and structure of intestinal mucosal flora. In addition, *Lactobacillus johnsonii* may be a characteristic species of Sishen Pill in treating diarrhea with kidney-yang deficiency syndrome.

1. Introduction

Sishen Pill is a classical typical prescription in traditional Chinese medicine (TCM) [1,2]. The prescription is based on Semen psoraleae and Semen myristicae, which are used to balance the spleen and kidney; Fructus evodiae expel cold, and Schisandra chinensis to astringent; ginger and jujube to harmonize the spleen and stomach, which are used together to restore kidney and spleen, reconstruction of intestinal mucosal flora to treat diarrhea [3–5]. However, the exact mechanism has not been fully elucidated.

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https://doi.org/10.1016/j.heliyon.2023.e16166
Received 8 December 2022; Received in revised form 24 April 2023; Accepted 8 May 2023
Available online 10 May 2023

The phyla Bacteroides and Firmicutes, as well as to a lesser extent the phyla Actinobacteria, Proteobacteria, Clostridium, and Verruciformes, dominate the intestinal flora of healthy adults. These organisms aid the body to maintain a relative balance and defend against infection by foreign pathogens [6]. In general, the body's intestinal mucosal flora in a dynamic equilibrium state, and the intricate interaction with the intestinal epithelial barrier and the local immune system aids in maintaining homeostasis and encouraging the proper growth of the intestinal tract [7]. Disturbance of the intestinal mucosal flora can harm intestinal mucosal cells, impair the effectiveness of the intestinal mucosal flora barrier, obliterate the mechanical and immune barriers of the intestinal mucosa, and result in a variety of gastrointestinal illnesses, including diarrhea and inflammatory bowel disease [8,9]. Traditional Chinese medicine (TCM) is multi-component, multi-target, and multi-modal in illness prevention and treatment, and as one of its main targets, the intestinal mucosal flora, interacts intricately and extensively with TCM [10] Studies using 16S amplicon sequencing to investigate the efficacy mechanisms of TCM and their constituents using intestinal flora as an entry route have also demonstrated that intestinal flora can respond to, mediate, and play a critical part in TCM against illnesses [11,12].

Based on the aforementioned investigations, we have put forth the hypothesis that Sishen Pill might be useful in treating diarrhea with kidney-yang deficiency syndrome by modulating the makeup and structure of intestinal mucosal flora. In order to find microbial markers that respond to the pharmacology of Sishen Pill and to evaluate the effectiveness of Sishen Pill on kidney-yang deficiency syndrome diarrhea from the perspective of the intestine, the study was to further investigate the effect of Sishen Pill on the intestinal mucosa microbial community during the treatment of diarrhea with kidney-yang deficiency syndrome diarrhea in mice by using 16S rRNA high-throughput sequencing . The experimental flow chart is shown in Fig. 1.

2. Materials and methods

2.1. Animal

Fifteen SPF-grade male Kunming mice, 4 weeks old, 18–22 g. Purchased from Hunan Slex Experimental Co Ltd. (license number: ZS-202105110016). All mice were kept in the Hunan University of Chinese Medicine's Experimental Animal Center (room temperature 23–25 °C, relative humidity 50–70%, and 12 h of light/darkness). All animal experiments were carried out in accordance with the guidelines approved by the Experimental Animal Management and Use Committee of Hunan University of Chinese Medicine (license number: SYXK[Xiang] 2019-0009). Animal experiments were approved by the Animal Ethics and Welfare Committee of Hunan University of Chinese Medicine (LLBH-202106120002) [13].

2.2. Medicine

We created an adenine suspension with a 5 mg/mL concentration by dilution with saline (Changsha Yaer Biological Co., Ltd., EZ2811A135). *Folium senna* decoction (Anhui Puren Herbal Beverage, 2005302) is prepared as follows: soaked in water for 20 min, boiled, and then placed over civil heat for 20 min. Once it has boiled, switch to civil heat for 20 min. After that, the medicinal liquid was filtered through gauze, the dregs were given a suitable amount of water, and the filtrate was prepared in the same manner. The two decoctions were then combined, amplified to a concentration of 1 g/mL, and stored at 4 °C refrigerator . The ingredients and dosage for the Sishen Pill decotion were prepared in accordance with the pharmacy textbook [14] and included *Psoralea corylifolia* L. (No: HY21012201), *Myristica fragrans* Houtt. (No: Xiang 20160111), *Euodia rutaecarpa (Juss.)* Benth. (No: 2020082804), *Schisandra chinensis (Turcz.)* Baill. (No: HY21020304), *Ziziphus jujuba* Mill. (No: 2103120082), *Zingiber officinale* Rose. (No: 170903), and all Chinese herbal decoction from Hunan Junhao Chinese Herbal Medicine Science and Trade Co., Ltd.

2.3. Reagent Kits

Na^+-K^+-ATP-ase ELISA Kit (Jiangsu Jingmei Biotechnology Co., LTD, number: JM-11845M1). Ca^{2+}-Mg^{2+}-ATP-ase ELISA Kit (Jiangsu Jingmei Biotechnology Co., LTD, number: JM-12156M1).

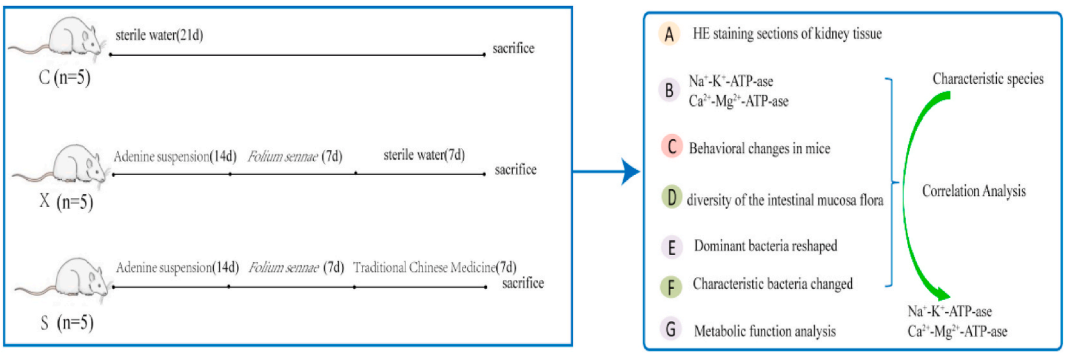

Fig. 1. Experimental flow chart.

2.4. Grouping and modeling of animals

Fifteen SPF-grade Kunming mice were fed adaptably for 3 d before being randomly assigned to Normal control group (C), Model self-healing group (X) and Sishen Pill group (S) using the random number table method, 5 mice/group, 5 mice/cage. Adenine suspension was gavaged to mice in groups X and S once daily for 14 d at a dose of 50 mg/(kg•d), 0.4 mL/each. From the 8th day, The X group was given *Folium senna* decoction by orogastric, 10 g/(kg•d), 0.4 mL/each, once/d for 7 d. Group C received the same amount of sterile water at the same time. The experimental mice in the X group terminated the experimental intervention after successful modeling and went back to drinking regular water. The S group mice were given the equivalent doses of human and mouse body surface area at a ratio of 5 g/(kg•d), 0.35 mL/each, 2 times/d for 7 d. The C group was gavaged with the same volume of sterile water as group X, 2 times/d for 7 d [15–17].

2.5. General behavioural observations

Throughout the modeling period, keep track of changes in the mice's body weight, food and water consumption, fecal features, mental state, activity, and physical symptoms [18].

2.6. Model evaluation criteria

The diagnostic criteria for macroscopic symptoms in mice with kidney-yang deficiency syndrome diarrhea were: dilute feces, or incomplete pellets, cold extremities, curved and arched back, decreased appetite and body weight, and depression. These symptoms were based on the clinical manifestations of kidney-yang deficiency syndrome diarrhea as well as pertinent references [19,20]. A solid foundation for model evaluation was offered based on macroscopic sign presentations and histological sections of the kidneys.

2.7. Pathological sectioning

The kidney tissues were dissected under aseptic conditions to remove any nodular tissue. The kidney tissues were then embedded in paraffin, fixed in 4% paraformaldehyde solution, dehydrated in gradient ethanol, transparent in xylene, stained with HE, and examined under light microscopy for histopathological changes in the kidney.

2.8. ELISA analysis

Whole blood specimens (0.7–1.5 mL) were collected from 1 cage (5 mice) in each group using plain serum tubes (red cap hoover blood collection tubes), centrifuged at 3000 r/min for 10 min to separate the serum, and the sample was packed into sterile centrifuge tubes. The ELISA blood samples were left at room temperature for 30 min. The blood samples used in the ELISA test were left at room temperature for 30 min. The serum was separated after centrifugation at 3000 r/min for 10 min, and the test substance was then placed into sterilized centrifuge tubes. To set up the plate layout, add samples, add enzymes, incubate, wash the plate, develop color, stop the reaction, and use machine detection, adhere to the instructions provided in the ELISA kit [21].

2.9. 16S rRNA gene high throughput sequencing

After the experiment, the mice were executed by cervical dislocation. The mice's abdominal cavity was opened, and the small intestine was taken out under aseptic circumstances. The intestinal mucosa was scraped off and collected on sterile coverslips after the small intestine was sliced along its long axis, cleaned with saline, and dried on filter paper [22]. For the purpose of identifying the intestinal mucosal flora, the samples were placed in 1.5 mL sanitized centrifuge tubes, given numbers and weights, and then kept in a −80 °C freezer. Using a bacterial DNA kit, total genomic DNA samples were isolated from the intestinal mucosa samples (OMEGA, USA). Thermo Fisher Scientific's NanoDrop NC2000 spectrophotometer and agarose gel electrophoresis were used to measure the quantity and quality of the isolated DNA. For the study of bacterial PCR results, the forward primer 27F (5′-AGAGAGTTTGATCMTGGCTCAG-3′) and the reverse primer 1492R (5′-GGACTACHVGGGTWTCTAAT-3′) were employed. The 16SrRNA gene was amplified to a length that was nearly full. Q5 high-fidelity DNA polymerase was used in the polymerase chain reaction (PCR) to amplify the 16S rRNA gene (New England BioLabs, USA). Electrophoresis on 2% agarose gel was used to identify the PCR results, and the Axygen® AxyPrep DNA Gel Extraction Kit was used to purify them. The recovered PCR amplification products were quantified fluorescently using the Quant-it PicoGreen dsDNA Detection Kit. Samples were blended in proportion to each sample's required sequencing based on the findings of the fluorescence quantification. Paiseno Biological Co., LTD carried out the sequencing (Shanghai, China) [23].

2.10. Bioinformatics and statistical analysis

High-throughput 16S rRNA sequencing was used to examine the gut mucosal microbiota, and sequences with similarity more than 97% were assigned to an OTU [24]. To test the depth of the sequencing and assess the quality of sequence data, species accumulation curves and dilution curves were utilized. Chao1 and Observed species indexes measure community abundance. Simpson and Shannon indices measure community diversity, and higher index values denote more community abundance and variety. Beta diversity analysis

examined the similarity of community structure among different samples. The community data structure was organically broken down using principal coordinate analysis (PCoA), non-metric multidimensional scaling (NMDS), and cluster analysis. To see how the samples varied from one another, the samples were ranked by Ordination Linear discriminant analysis Effect Size (LEfSe) [25] and receiver operating characteristic curve (ROC) detected groups with differences in intestinal mucosal abundance and identified potential biomarkers [26,27]. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) was used to perform functional unit PCoA and Kyoto Encyclopedia of Genes and Genomes (KEGG) database analysis for prediction of colony function. The relationship between biochemical markers and intestinal mucosal flora was investigated using Scatter plot of Spearman correlation coefficient.

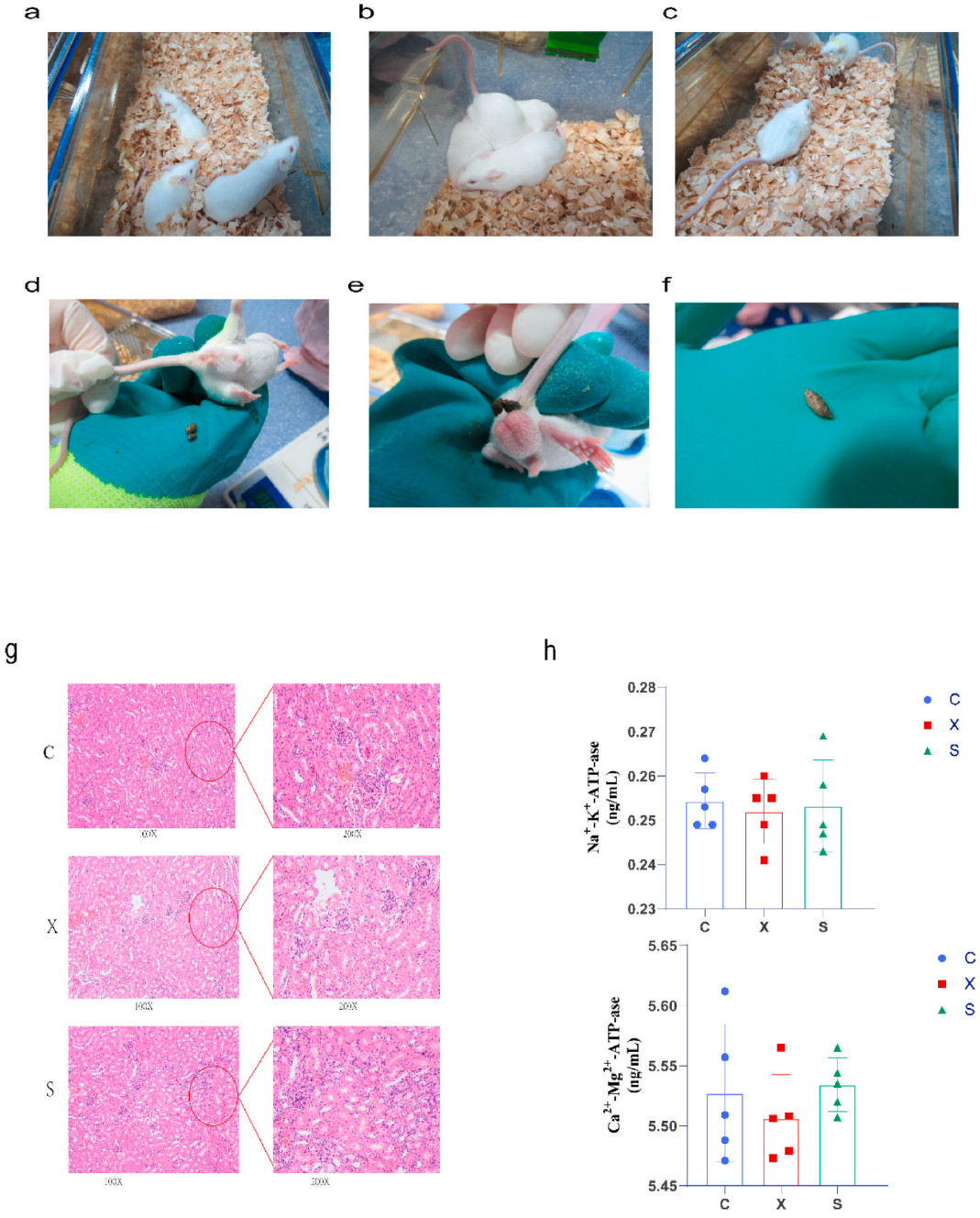

Fig. 2. Behavioral changes in mice. (a) Mental state of mice in the C group. (b) Mental state of mice in the X group. (c) Mental state of t mice in the S group. (d) Feces of mice in the C group. (e) Feces of mice in the X group. (f) Feces of the mice in the S group. (g) HE staining sections of kidney tissue. (h) Energy metabolism analysis in mice. C: Control group (n = 5); X: Model self-healing group (n = 5); S: Sishen Pill group (n = 5). The values were expressed as mean ± standard deviation.

The data collected from each group were reported as mean standard deviation using SPSS 21.00 program for statistical analysis. One-way ANOVA was employed and the Tukey HSD method was utilized for multiple comparisons between groups if the data from the three groups fit the normal distribution and the variance was chi-square. Wilcoxon rank sum test was applied if the data did not follow a normal distribution and the variances were not homogeneous. $P < 0.05$ indicates a statistical difference, $P < 0.01$ indicates a very strong statistical difference, otherwise there was no statistical significance [28].

3. Results

3.1. Behavioral, kidney structure, energy metabolism changed in mice

The mice in the C group displayed normal mental status, autonomous behaviors, smooth fur, and responsiveness (Fig. 2 a, d). Poor mental health, thin and dull fur, and loose and unformed feces were also characteristics of the X group (Fig. 2 b, e). The mice in the S group gradually resumed their regular behavior and formed feces (Fig. 2 c, f).

In Fig. 2 g, the kidney units of the C group showed normal structural morphology and no abnormal pathological symptoms, whereas the X group had glomerular thylakoid hyperplasia, interstitial edema and congestion, inflammatory cell aggregation, tubular dilatation of varied degrees, lumen enlargement, and tubular wall degradation and edema. The fact that the S group kidney structure tended to be

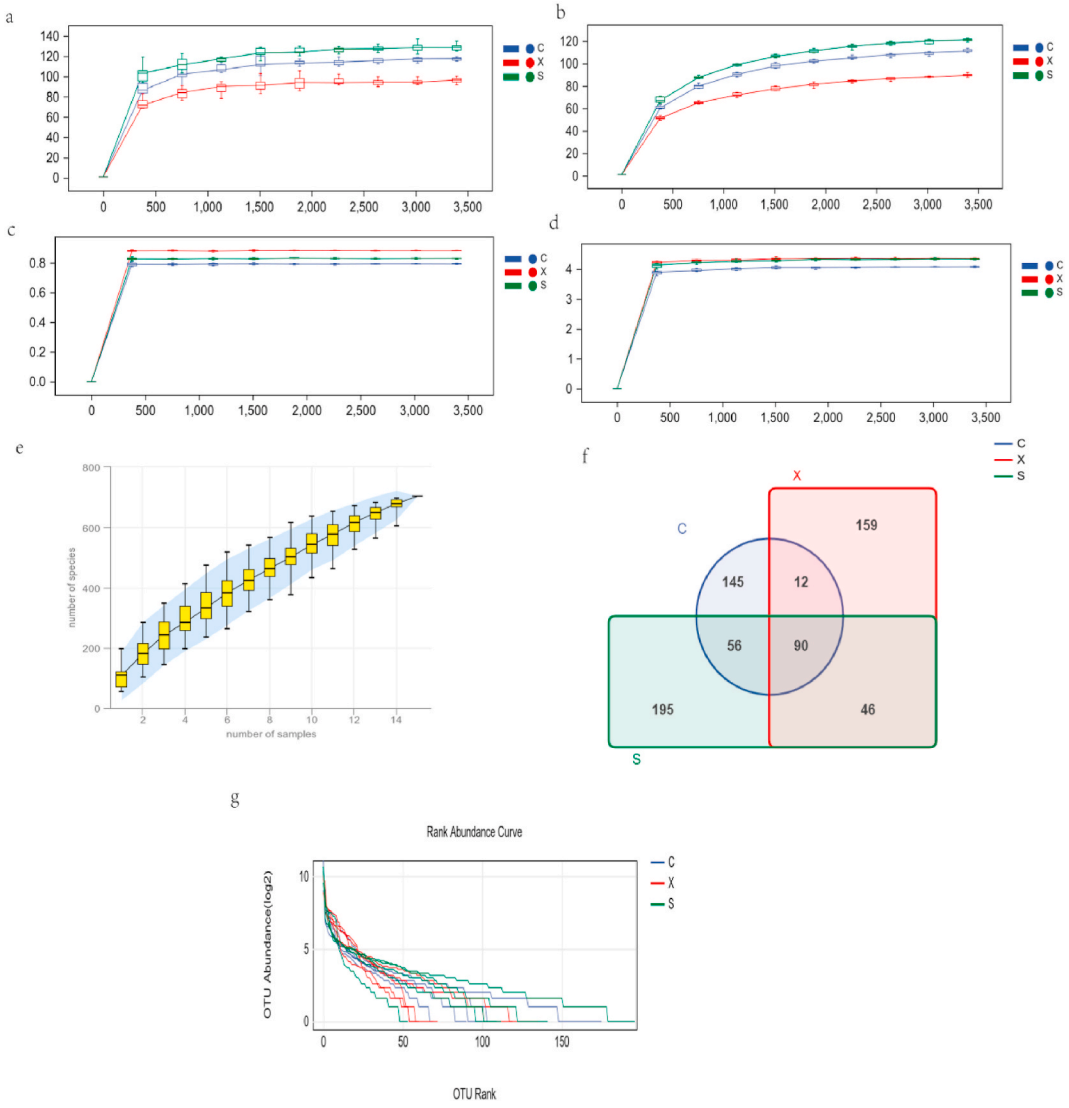

Fig. 3. Sequencing data quality assessment and OTU counts of intestinal mucosal flora. (a) Dilution curves of Chao1. (b) Dilution curves of Observed_species. (c) Dilution curves of Simspon. (d) Dilution curves of Shannon. (e) Species accumulation curves. (f) Venn diagram. (g) Distribution curves of OTU class abundance. C: Control group (n = 5); X: Model self-healing group (n = 5); S: Sishen Pill group (n = 5).

normal, with intact structure and tight cell organization, compared to the X group, suggests that Sishen Pill had a promoting effect on the recovery of kidney structure in mice.

Fig. 2 h demonstrates that the S group had stronger Na^+-K^+-ATP-ase and Ca^{2+}-Mg^{2+}-ATP-ase activities than the X group ($P > 0.05$; $P > 0.05$). Compared to the C group, the S group Na^+-K^+-ATP-ase activity reduced while the Ca^{2+}-Mg^{2+}-ATP-ase activity increased ($P > 0.05$; $P > 0.05$). The outcomes suggested that Sishen Pill treatment improved energy metabolism in diarrhea mice with kidney-yang deficiency syndrome diarrhea.

3.2. *Intestinal mucosal flora in mice sequencing data quality assessment and OTU count*

The dilution curve initially showed an inflection point, then the curve flattened with increasing sequencing depth and reached a plateau, showing that the sequencing depth of the three sets of samples was reasonable enough to cover the majority of biological species and the species richness of the test samples was sufficient for further research (Fig. 3 a-d). The species accumulation curve is shown in Fig. 3 e. The number of detected species increased significantly as sample size was increased, and the curve was relatively steep. However, after a certain point, further increases in sample size stopped yielding new species detections, and the curve flattened, indicating that the sample size was adequate to reflect the richness of the community.

The OTU Venn diagram was used to assess the uncommon or common OTUs found in various sample groups and to show how similar and distinct the samples were at OTU level. In this experiment, sequences with similarity more than 97% were classified into one OTU. After removing rare OTUs and leveling the data from the sampling process, an abundance matrix with 715 OTUs was obtained for the examination of microbial diversity makeup. There were 387 OTUs in the S group, 303 OTUs in the C group, and 307 OTUs in the X group. Approximately 12.59% (90/715) OTUs were shared by the three groups, and the characteristic OTUs in groups the C, X, and S group accounted for 20.28% (145/715), 22.24% (159/715), and 27.27% (195/715) (Fig. 3 f). Further investigation revealed that group C shared 102 and 146 OTUs with groups X and S, respectively. The aforementioned findings demonstrated that the S group had a larger microbiological richness than the X group, comparable to the C group, and established a distinction from the X group. This indicated that Sishen Pill intervention altered microbial population of intestine mucosa in diarrhea mice with kidney-yang deficiency syndrome.

After that, we used abundance log2 values to create rank abundance distribution curves to examine the community structure of each group intestinal mucosal flora (Fig. 3 g). The species richness and resource distribution can be inferred from the rank abundance distribution curves. For each sample, the OTU level and abundance are shown by the horizontal and vertical coordinates, respectively. They stand for variations in the relative abundance of OTUs among the three groups. There are more OTUs in the sample the longer fold line is. The homogeneity of the group is inversely correlated with the smoothness of slope and steepness of fold line. According to Fig. 3

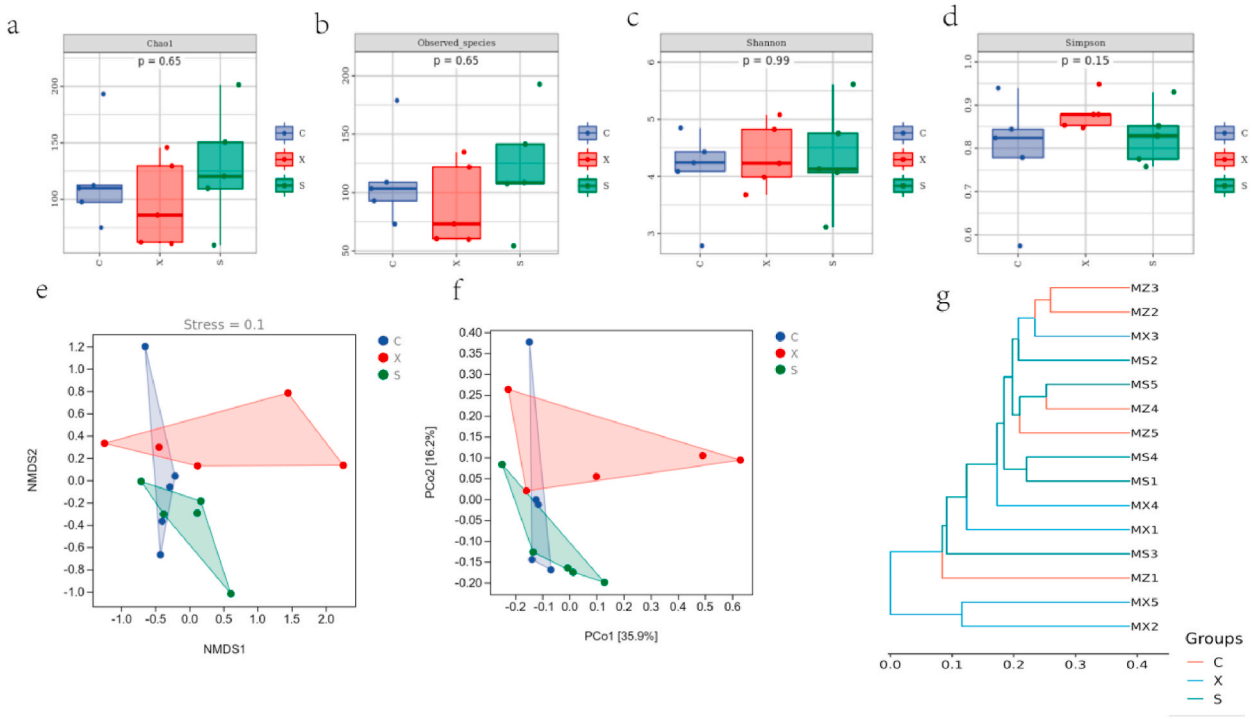

Fig. 4. Effect of Sishen Pill on the diversity of intestinal mucosal flora in mice. (a) Chao1 index. (b) Observed_species index. (c) Shannon index. (d) Simpson index. (e) NMDS analysis. (f) PCoA analysis. (g) Cluster analysis. C: Control group (n = 5); X: Model self-healing group (n = 5); S: Sishen Pill group (n = 5).

g, which is in line with the OTU report above, the Sishen Pill intervention group had the greatest number of OTUs.

3.3. Sishen Pill increased intestinal mucosal flora diversity in mice

We calculated the Alpha diversity indices for the two groups of gut contents samples to see if there were any differences in the microbial communities diversity between the samples (Fig. 4 a-d). Among these, the Chao1 and Observed_species index concentrated on expressing the richness of the community, and the Shannan and Simpson index portrayed the homogeneity of the community. These findings demonstrated that the C group had the highest microbial richness, followed by group S ($P > 0.05$; $P > 0.05$). Group X had the most evenly distributed members, whereas groups C and S had similar levels ($P > 0.05$; $P > 0.05$). In conjunction with the Venn analysis results, it demonstrated that Sishen Pill had a moderating influence on the microbial diversity of intestinal mucosa in diarrhea mice with kidney-yang deficiency syndrome. Afterwards, we conducted the Beta diversity study to compare and contrast the community structures of the various groupings (Fig. 4 e-g). The bray_curtis distance matrix-based NMDS analysis (Fig. 4 e, f) revealed that the small intestine microbial communities in groups S and X had varied structural distribution traits. PCoA analysis using the bray_curtis distance matrix (Fig. 6 f) revealed that the confidence triangle of the S group microbial community was separated from that of the X group along the first coordinate axis. It demonstrated that Sishen Pill may, to some extent, restore and modify the Beta diversity of intestinal mucosa microbes in diarrhea mice with kidney-yang deficiency syndrome. It is clear from the clustering analysis (Fig. 4 g) that the C group has less intra-group variability since the distances between the samples are relatively short. Group X samples are substantial and clearly distinguished from group C samples. With the exception of sample S3, several samples from group S can be neatly clustered together. Additionally, S2 and S5 cluster better with the C group, indicating that group X had higher intra-group variability than groups C and S. In summary, Sishen Pill improved the diversity and architecture of intestinal mucosal flora.

3.4. Sishen Pill remodeled the dominant bacterial composition of intestinal mucosal flora in mice

The classification of Phylum, Genus, and Species for OTU was done independently, and the analysis of the dominant flora at each taxonomic level that had relative abundance more than 1% was done (Fig. 5 d). According to Fig. 5 a, the relative abundance of Firmicutes increased from 73.53% to 74.97% and then slightly dropped to 74.52% after Sishen Pill treatment. The relative abundance of Bacteroidetes increased marginally to 21.46% in the C group from 21.38% in the X group to 18.93% in the S group. Proteobacteria

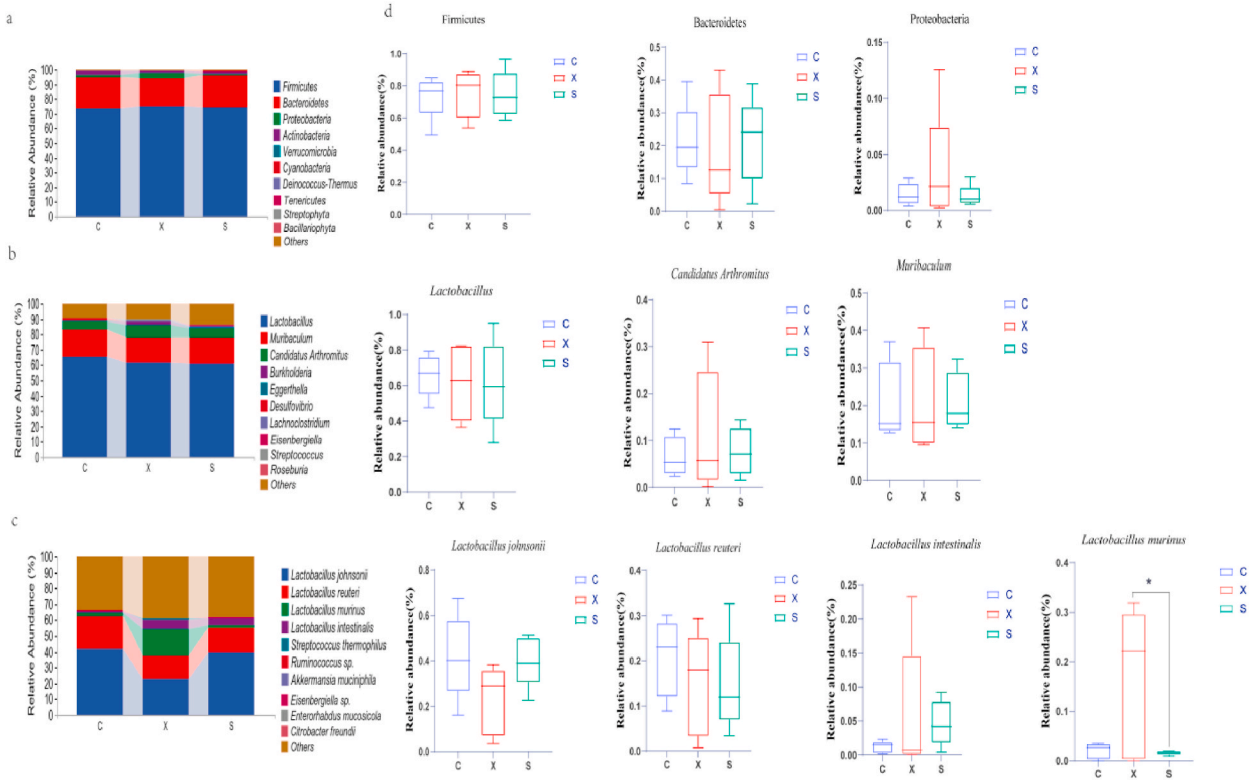

Fig. 5. Effect of Sishen Pill on relative abundance of gut mucosal microbiota in mice. (a) Relative abundance of phylum level of intestinal mucosal flora. (b) Relative abundance of genus level of intestinal mucosal flora. (c) Relative abundance of species level of intestinal mucosal flora. (d) Phylum, genus and species levels of dominant bacteria of intestinal mucosal flora in mice. C: Control group (n = 5); X: Model self-healing group (n = 5); S: Sishen Pill group (n = 5). *indicates $P < 0.05$.

relative abundance rose from 1.45% to 3.53% before Sishen Pill involvement, when it fell to 1.28%. The differences were not statistically significant.

The small intestinal mucosal microbiota of all three groups of experimental mice was primarily made up of the genus-level organisms *Lactobacillus*, *Muribaculum*, and *Candidatus-Arthromitus* (Fig. 5 b). The proportions in groups C, X, and S were: *Lactobacillus* (65.78% vs 61.40% vs 61.17%), *Candidatus-Arthromitus* (5.10% vs 8.5% vs 6.12%), *Muribaculum* (17.44% vs 16.25% vs 16.65%).

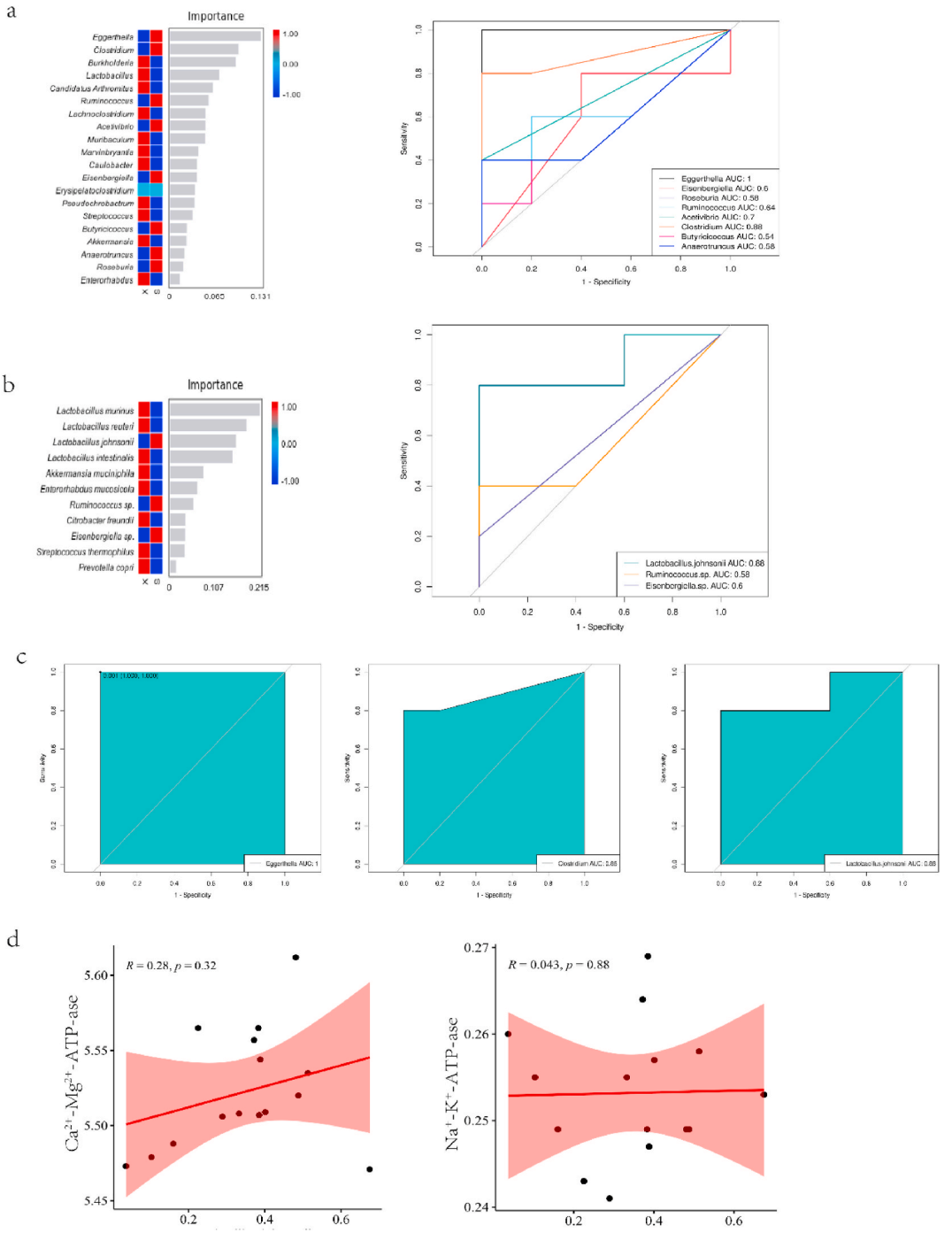

Fig. 6. Analysis of the characteristic bacteria of intestinal mucosal flora. (a) Random forest diagram and ROC curve of genus level. (b). Random forest diagram and ROC curve of species level. (c) ROC curve of *Clostridium*, *Eggerthella*, *Lactobacillus johnsonii*. The larger the area under the curve, the higher the diagnostic accuracy. (d) Scatter plot of *Lactobacillus johnsoni* with Na^{+} -K^{+}-ATP-ase and Ca^{2+}-Mg^{2+}-ATP-ase Spearman correlation coefficients. C: Control group (n = 5); X: Model self-healing group (n = 5); S: Sishen Pill group (n = 5).

Statistics did not support the discrepancies. *Burkholderia* was present only in group X, accounting for 2.42%, and none in groups C and S.

The main species (relative abundance>1%) in groups C, X, and S were all *Lactobacillus johnsonii* (41.77% vs. 22.86% vs. 39.99%, $P > 0.05$), *Lactobacillus reuteri* (20.77% vs. 14.97% vs. 14.86%, $P > 0.05$), *Lactobacillus intestinalis* (1.20% VS 6.02% VS 4.72%, $P > 0.05$), *Lactobacillus murinus* (2.04% vs. 16.41% vs. 1.58%), where *Lactobacillus murinus* was significantly higher in the X group than in the S group ($P < 0.05$). According to the information above, Sishen Pill interventional therapy can enhance the makeup of intestinal mucosal flora (Fig. 5 c).

3.5. Sishen Pill affected the interaction between characteristic species of intestinal mucosal flora and energy metabolism in mice

Random forest analysis revealed that the S group and the X group had significantly different relative abundances of mucosal flora. Compared with group X; the characteristic genera (Fig. 6 a) were *Eggerthella*, *Clostridium*, *Ruminococcus*, *Acetivibrio*, *Eisenbergiella*, *Butyricicoccus*, *Anaerotruncus*, *Roseburia*; the characteristic species (Fig. 6 b) were *Lactobacillus johnsonii*, *Ruminococcus* sp., and *Eisenbergiella* sp. AUC values for *Lactobacillus johnsonii* (AUC = 0.88), *Clostridium* (AUC = 0.88), and *Eggerthella* (AUC = 1) were all quite high, which can be used as a major characteristic bacteria to separate the S group from the X group, according to ROC analysis (Fig. 6 c).

Lactobacillus johnsonii, as a crucial characteristic species to distinguish group S from group X. It was favorably connected with Na^+-K^+-ATP-ase and Ca^{2+}-Mg^{2+}-ATP-ase, according to the Spearman correlation coefficient, and none of the differences were statistically significant (Fig. 6 d). It suggests that the interplay of the aforementioned elements may have contributed to the increase in energy

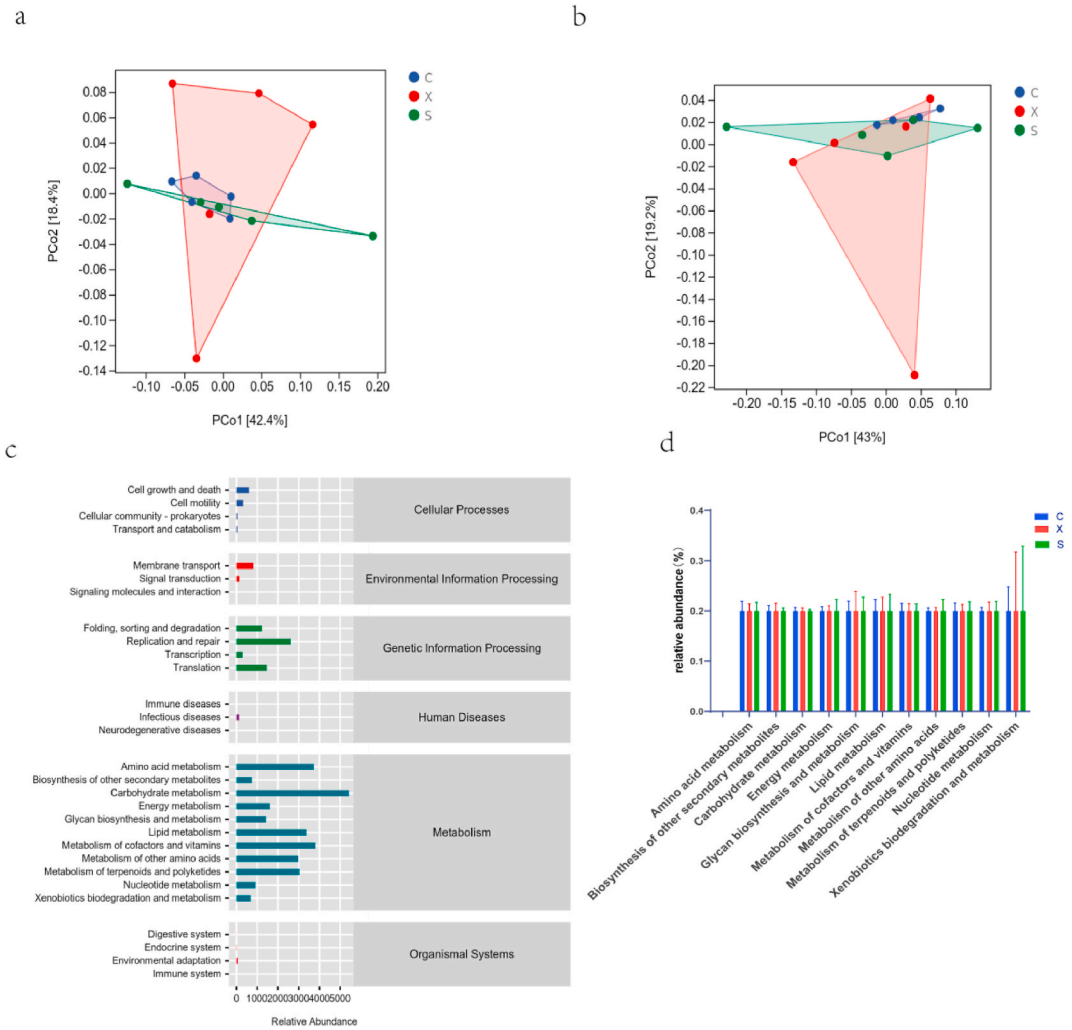

Fig. 7. Functional analysis of intestinal mucosal flora. (a) MetaCyc functional unit PCoA map. (b) PCoA diagram of KEGG functional units. (c) Predicted abundance of KEGG function. (d) Functional analysis of metabolic pathways in different treatment groups. C: Control group (n = 5); X: Model self-healing group (n = 5); S: Sishen Pill group (n = 5).

metabolism and the restoration of intestinal mucosa in mice treated with Sishen Pill for kidney-yang deficient diarrhea.

3.6. Sishen Pill altered the function of intestinal mucosal flora in mice

We carried out functional unit PCoA analysis, mapping of flora composition data to KEGG database for prediction of flora function using PICRUSt, and correlation analysis of small intestine flora composition and functional pathways in order to better understand the role of intestinal mucosal flora in the treatment of diarrhea with kidney yang deficiency by Sishen Pill. The study indicate that while samples from the three databases did not statistically differ from groups C and S or from each other, they did all significantly differ from group X. (Fig. 7 a, b). Meanwhile, intestinal mucosal flora functions were generally classified into six categories, and the second level included 29 subfunctional categories, of which metabolic functions accounted for a greater abundance. Significant functions in controlling energy metabolism, amino acid metabolism, and carbohydrate metabolism were played by intestinal mucosal flora (Fig. 7 c).

Further metabolic pathway study revealed that intestinal mucosa flora mostly performed the roles of glucose metabolism, amino acid metabolism, and lipid metabolism (Fig. 7 d). In the S group compared to the X group, the relative abundance of energy metabolism, lipid metabolism, metabolism of other amino acids, exogenous biodegradation, and metabolism dropped while that of other secondary metabolite biosynthesis, carbohydrate metabolism, and glycan biosynthesis increased. In contrast to the C group, the S group showed a greater relative abundance of amino acid metabolism and energy metabolism. None of the differences were statistically significant.

4. Discussion

The majority of ancient and contemporary physicians think that spleen, liver, kidneys, and other internal organs, is the primary internal organ of diarrhea. For this reason, treating diarrhea should not should not ignore restoring kidney function. Sishen Pill is one of the classic formulas for the treatment of diarrhea with spleen and kidneys deficiency. The study found that the kidney structure of mice with kidney-yang deficient diarrhea was clearly affected, as evidenced by glomerular and tubular damage, interstitial edema, and inflammatory cell aggregation [29,30]. Additionally, mice with kidney and spleen-yang deficiency diarrhea had significantly higher levels of mitochondrial uncoupling protein 2 (UCP2) in their kidneys, which increased their H^+ leak rate and reduced ATP synthesis. After Ershen Pill treatment, UCP2 was significantly improved and regulated [31]. Ca^{2+}-Mg^{2+}-ATP-ase and Na^+-K^+-ATP-ase are significant indicators of the level of human energy metabolism [32]. In mice with kidney-yang deficiency syndrome diarrhea, it was discovered that the activity of hepatic Na^+-K^+-ATP-ase and Ca^{2+}-Mg^{2+}-ATP-ase was greatly reduced, as well as the levels of cAMP (Cyclic adenosine monophosphate) and cAMP/cGMP(cyclic guanosine monophosphate) were dropped and cGMP was elevated, and improved following treatment with Sishen Pill [33,34]. In terms of energy metabolism in our study, restoration of kidney structure and an improvement in serum Na^+-K^+-ATP-ase and Ca^{2+}-Mg^{2+}-ATP-ase activities following Sishen Pill intervention. These findings are in line with earlier research. So, we conclude that Sishen Pill benefits involves controlling energy metabolism. Another topic worth looking into is Sishen Pill impact on kidney mitochondrial.

The physiopathology of the gut is strongly tied to the composition of the intestinal flora, which is made up of billions of commensal microorganisms that are essential for sustaining the proper metabolism and physiological function [35,36]. TCM has been proven to be more helpful in treating diarrhea and can affect the composition and metabolic activity of intestinal flora through interaction with intestinal microorganisms, achieving therapeutic goals or changing the efficacy of treatment [37]. Sishen Pill regulates the balance of intestinal microbes, which can have an impact on the development and course of disease [1,38]. According to our experiment results, the S group had a higher number of OTU in intestinal mucosa flora than the X group; the S group also had restored levels of Alpha and Beta diversity compared to the X group; the S group flora distribution was clearly different from the X group. The information above suggests that Sishen Pill can modify the diversity and composition of intestinal mucosal flora in diarrhea kidney-yang deficiency mice.

We can better understand how Sishen Pill alters the intestinal microbial environment by contrasting the numerous changes in intestinal mucosal flora between the X and S groups. Evodiamine (Evo) is an important component of Fructus evodiae. It has been found that Evodiamine can reduce the abundance of *Bifidobacterium*, *Parabacteroides*, *Mucispirillum*, and *Turicibacter* and increase the relative abundance of *Bacillus*, *Lactobacillus*, and *Rumenococcus* in colitis-prone animals [39]. Study also revealed that the Ershen Pill group compared with the normal group had an increased abundance of *Bifidobacterium* and *Lactobacillus* and decrease *Escherichia coli* and *Enterococcus* in the intestine [31]. Sishen Pill was found to enhance the abundance of *Bifidobacterium*, *Lactobacillus*, and *Bacteroidetes* while decreasing the abundance of *Enterobacter* and *Enterococcus* [40]. By decreasing the quantity of Proteobacteria and *Mycoplasma* and increasing the abundance of *Clostridium*, *Bacillus*, and *Romboutsia*, Sishen Pill can protect the intestinal mucosa [17,38]. It is thought that this remedy can aid in restoration of the intestinal flora variety. In terms of intestinal mucosal flora in our study, in the S group, Firmicutes was increased, while Bacteroidetes and Proteobacteria were significantly reduced and the ratio of the Firmicutes to Bacteroidetes the (F/B value) was also near the C group. The F/B value, a conventional dysbiosis measure, offers additional proof that Sishen Pill reverses intestinal mucosal dysbiosis in mice with kidney-yang deficiency syndrome diarrhea [41,42] A moderate enrichment of *Lactobacillus johnsonii* was identified in S group compared with X group, suggesting a protective effect of Sishen Pill on *Lactobacillus johnsonii*. It has been demonstrated that *Lactobacillus johnsonii* is a helpful bacterium with anti-inflammatory characteristics that reduce systemic pro-inflammatory immune responses, and can increase mitochondrial levels and activity while promoting lipolysis [43–45]. This showed that Sishen Pill regulated the balance of gut microbiota and may reduce intestinal inflammation. The characteristic flora between groups X and S differed significantly in the random forest study. *Lactobacillus johnsonii* was a significant marker species for group differences according to ROC analysis. Spearman correlation coefficient revealed a favorable link between

Lactobacillus johnsonii and both Ca^{2+}-Mg^{2+}-ATP-ase and Na^{+}-K^{+}-ATP-ase. Therefore, We propose that by raising the abundance of *Lactobacillus johnsonii*, Sishen pills therapy for diarrhea may decrease intestinal inflammation and enhance energy metabolism. We next investigated at the functional study of the metabolic pathways and discovered that Sishen Pill may mediate the treatment by impacting energy metabolism, lipid metabolism, metabolism of other amino acids, xenobiotics biodegradation and metabolism in mice. The information above suggests that Sishen Pill has a significant impact on the intestinal mucosa flora. In conclusion, a crucial step will be to analyze the typical intestinal microbes and the way TCM works [22,46–48].

5. Conclusions

In conclusion, Sishen Pill improves the variety and structure of intestine mucosa microbial community of mice with kidney-yang deficiency syndrome diarrhea, drastically reducing the abundance of *Lactobacillus murinus*, and adjusts the abundance of other flora, normalizing the ratio of F/B, and affecting the activities of Na^{+}-K^{+}-ATP-ase and Ca^{2+}-Mg^{2+}-ATP-ase to improve energy metabolism. Furthermore, a synergistic impact on energy metabolism was found to exist between the Sishen Pill intervention treatment and *Lactobacillus johnsonii*(Fig. 8). Investigating the impact of the one medication that is crucial in Sishen Pill prescription on the intestinal mucosal flora of kidney-yang deficiency syndrome diarrhea mice is worthwhile.

Funding statement

Zhoujin Tan was supported by the Natural Science Foundation of Hunan Province [2022JJ30440] and Key Scientific Research Project of Hunan Provincial Education Department [22A0270].

Institutional animal care and use committee statement

This study was approved by Animal Ethics and Welfare Committee of Hunan University of Chinese Medicine. All authors knew and approved of this animal experiment.

Author contribution statement

Jiayuan Zhu: Conceived and designed the experiments, Performed the experiments, Wrote the paper.
Xiaoya Li, Kang Zhou: Conceived and designed the experiments, Analyzed and interpreted the data.
Na Deng, Bo Qiao: Performed the experiments.
Zhoujin Tan, Dandan Li: Contributed reagents, materials, analysis tools or data, Conceived and designed the experiments.

Data availability statement

Data associated with this study has been deposited at The data underlying this study is available within the manuscript. The gut mucosal microbiota sequencing data has been uploaded to the NCBI database (https://www.ncbi.nlm.nih.gov/), no. PRJNA854687.

Additional information

No additional information is available for this paper.

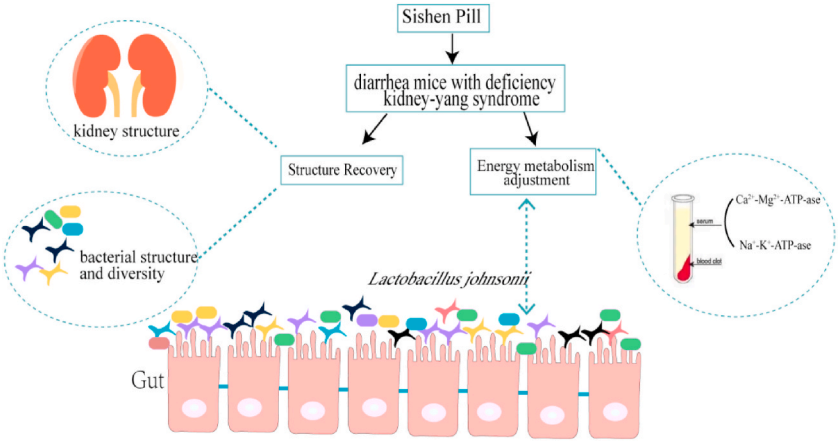

Fig. 8. Interaction between "bacteria-Energy metabolism-kidney structure" during remediation process of diarrhea with deficiency kidney-yang syndrome in Sishen Pill treatment.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

References

[1] X.Y. Zhang, Y.N. Xu, H.M. Zhao, R.Y. Liu, K. Ding, Compatibility significance of Psoralea fruit with nutmeg as core medical com-binations of Sishen pill via theoretical proof, Chin. Arch. Tradit. Chin. Med. 37 (2019) 1660–1662, https://doi.org/10.13193/j.issn.1673-7717.2019.07.030.

[2] R.S. Wang, Z.T. Zhang, W.W. Chao, W.T. Chen, J.S. Zhang, Mechanism of baiyaojian in treating ulcerative colitis based on intestinal flora and intestinal mucosal barrier function, Chin. J. Exp. Tradit. Med. Formulae 27 (2021) 46–54, https://doi.org/10.13422/j.cnki.syfjx.20211347.

[3] X.Y. Lin, N. Deng, Y. Cai, F. Liu, Effects of Sishen pills on ghrelin-CaM-MLCK Signaling pathway in Sinuses ventriculi of rats with diarrhea of yang deficieney of spleen and kidney, Chin. J. Inf. Tradit. Chin. Med. 24 (2017) 34–37.

[4] X.Y. Li, Z.M. Wang, X.H. Yao, S.Z. Li, J.P. Cui, H.B. Xiao, W.Y. Niu, Research progress of pharmacological effects and clinical application of Sishen pill, J. Liaoning Univ. Tradit. Chin. Med. 23 (2021) 122–126, https://doi.org/10.13194/j.issn.1673-842x.2021.04.027.

[5] N.L. Li, J.P. Gao, L.Z. Li, X.D. Zhu, D. Wang, Y.Q. Liu, M.Q. Xun, Effect of SiShen Pill on Th1/Th2 immune balance in rats with ulcerative colitis model of spleen and kidney Yang deficiency, Henan. Tradit. Chin. Med. 28 (2018) 1501–1505, https://doi.org/10.16367/j.issn.1003-5028.2018.10.0403.

[6] Y. Ran, M.F. Xiao, S.W. Chen, J.Y. Shao, Z.N. Lei, J.R. Li, Y.C. Li, Research progress of gut microbiota and inflammatory bowel disease, Chin. J. Gastroenterol. Hepatol. 30 (2021) 6–9.

[7] S. Haq, J. Grondin, S. Banskota, W.I. Khan, Autophagy: roles in intestinal mucosal homeostasis and inflammation, J. Biomed. Sci. 26 (2019) 1–14, https://doi.org/10.1186/s12929-019-0512-2.

[8] Y.X. Li, S.T. Xia, X.H. Jiang, C. Feng, S.M. Gong, J. Ma, Z.F. Fang, J. Yin, Y.L. Yin, Gut microbiota and diarrhea: an updated review, Front. Cell. Infect. Microbiol. 11 (2012), 625210, https://doi.org/10.3389/fcimb.2021.625210.

[9] A. Altomare, C.D. Rosa, E. Imperia, S. Emerenziani, M. Cicala, M.P.L. Guarino, Diarrhea predominant-irritable bowel syndrome (IBS-D): effects of different nutritional patterns on intestinal dysbiosis and symptoms, Nutrients 13 (2021) 1506, https://doi.org/10.3390/nu13051506.

[10] T.T. Zhao, Z. Wang, Z.L. Liu, Y.H. Xu, Pivotal role of the interaction between herbal medicines and gut microbiota on disease treatment, Curr. Drug Targets 22 (2020) 336–346, https://doi.org/10.3389/fmicb.2021.626691.

[11] Q.M. Li, Y. Cui, B.C. Xu, Y.H. Wang, F.F. Lv, Z. Li, H.A. Li, X.G. Chen, X.M. Peng, Y.T. Chen, E.Y. Wu, D.S. Qu, Y.C. Jian, H.B. Si, Main active components of Jiawei Gegen Qinlian decoction protects against ulcerative colitis under different dietary environments in a gut microbiota-dependent manner, Pharmacol. Res. 170 (2021), 105694, https://doi.org/10.1016/j.phrs.2021.105694.

[12] J.X. Tian, B.B. Bai, Z.Z. Gao, Y.Y. Yang, H.R. Wu, X.M. Wang, J. Wang, M. Li, X.L. Tong, Alleviation effects of GQD, a traditional Chinese medicine formula, on diabetes rats linked to modulation of the gut microbiome, Front. Cell. Infect. Microbiol. 11 (2021) 1–11, https://doi.org/10.3389/fcimb.2021.740236.

[13] Y. Wu, X.X. Peng, X.Y. Li, D.D. Li, Z.J. Tan, R. Yu, Sex hormones influences intestinal microbiota composition in mice, Front. Microbiol. 13 (2022), 964847, https://doi.org/10.3389/fmicb.2022.964847.

[14] C.Y. Gao, J. Li, The origin of Sishen Pill formula, Acta Chin. Med. Pharmacol. 39 (2011) 147–149, https://doi.org/10.19664/j.cnki.1002-2392.2011.04.067.

[15] C.Y. Zhang, H.Q. Shao, D.D. Li, N.Q. Xiao, Z.J. Tan, Role of tryptophan-metabolizing microbiota in mice diarrhea caused by *Folium sennae* extracts, BMC Microbiol. 20 (2020) 185, https://doi.org/10.1186/s12866-020-01864-x.

[16] X.Y. Li, J.Y. Zhu, Y. Wu, Y.W. Liu, H.Y. Hui, Z.J. Tan, Study on the adenine combined with *folium sennae* on the diarrhea mice with deficiency kidney-yang syndrome using testing syndromes with prescriptions, J. Tradit. Chin. Med. 63 (2022) 1368–1373, https://doi.org/10.13288/j.11-2166/r.2022.14.012.

[17] J.X. Liu, Y.L. Wang, Y. Li, D.X. Zhou, D.F. Wang, X.R. Ma, H.X. Song, W.P. Yang, H.N. Wang, Experimental study on the effect of Sishen Wan on intestinal flora in rats with diarrhea-type irritable bowel syndrome, Acta Pharm. Sin. 54 (2019) 670–677, https://doi.org/10.16438/j.0513-4870.2018-0920.

[18] X.Y. Li, N. Deng, T. Zheng, B. Qiao, M.J. Peng, N.Q. Xiao, Z.J. Tan, Importance of Dendrobium officinale in improving the adverse effects of high-fat diet on mice associated with intestinal contents microbiota, Front. Nutr. 9 (2022), 957334, https://doi.org/10.3389/fnut.2022.957334.

[19] Z.Y. Shen, Reference standards for identifying deficiency in Chinese medicine, Chin. J. Integr. Tradit. West. Med. 3 (1983) 117.

[20] S.S. Zhang, C.J. Wang, Y.F. Li, N. Wang, Consensus opinion on TCM diagnosis and treatment of diarrhea, J. Tradit. Chin. Med. 58 (2017) 1256–1260, https://doi.org/10.13288/j.11-2166/r.2017.14.023.

[21] B. Qiao, J. Liu, N. Deng, Y. Cai, Y. Bian, Y.Y. Wu, Z.J. Tan, Gut content microbiota dysbiosis and dysregulated lipid metabolism in diarrhea caused by high-fat diet in a fatigued state, Food Funct. (2023), https://doi.org/10.1039/d3fo00378g.

[22] X.Y. Li, X.X. Peng, K.X. Guo, Z.J. Tan, Bacterial diversity in intestinal mucosa of mice fed with Dendrobium officinale and high-fat diet, 3 Biotech 11 (2021) 1–12, https://doi.org/10.1007/s13205-020-02558-x.

[23] Y.S. He, Y. Tang, M.J. Peng, G.Z. Xie, W.G. Li, Z.J. Tan, Influence of Debaryomyces hansenii on bacterial lactase gene diversity in intestinal mucosa of mice with antibiotic-associated diarrhea, PLoS One 14 (2019), e0225802, https://doi.org/10.1371/journal.pone.0225802.

[24] W.Y. Wang, A. Srivathsan, M. Foo, S.K. Yamane, R. Meier, Sorting specimen-rich invertebrate samples with cost-effective NGS barcodes: validating a reverse workflow for specimen processing, Mol. Ecol. Resour. 18 (2018) 490–501, https://doi.org/10.1111/1755-0998.12751.

[25] F. Chang, S.S. He, Y.D. Chen, Assisted Selection of biomarkers by linear discriminant analysis effect size (LEfSe) in microbiome data, J. Vis. Exp. (2022), https://doi.org/10.3791/61715.

[26] G.F. Fu, X.T. Dai, Y. Liang, Functional random forests for curve response, Sci. Rep. 11 (2021), 24159, https://doi.org/10.1038/s41598-021-02265-4.

[27] R.C. Edgar, UPARSE: Highly accurate OTU sequences from microbial amplicon reads, Nat. Methods 10 (2013) 996–998, https://doi.org/10.1038/nmeth.2604.

[28] C.Y. Zhang, X.X. Peng, H.Q. Shao, X.Y. Li, Y. Wu, Z.J. Tan, Gut microbiota comparison between intestinal contents and mucosa in mice with repeated stress-related diarrhea provides novel insight, Front. Microbiol. 12 (2021), 626691, https://doi.org/10.3389/fmicb.2021.626691.

[29] X.Y. Li, X.X. Peng, B. Qiao, M.J. Peng, N. Deng, R. Yu, Z.J. Tan, Impairment process of adenine combined with *Folium sennae*-induced diarrhea: association with interactions between Lactobacillus intestinalis, Bacteroides acidifaciens and acetic acid, inflammation, and kidney function, Cells 11 (2022) 3261, https://doi.org/10.3390/cells11203261.

[30] M. Sueyoshi, M. Fukunaga, M. Mei, A. Nakajima, G. Tanaka, T. Murase, Y. Narita, S. Hirata, D. Kadowaki, Effects of lactulose on renal function and gut microbiota in adenine-induced chronic kidney disease rats, Clin. Exp. Nephrol. 23 (2019) 908–919, https://doi.org/10.1007/s10157-019-01727-4.

[31] Z.M. Chen, X. Pan, M. Zhang, R. Xiong, Y.Y. Cui, L. Hu, C.J. Hu, Effect of Chinese medicine in ershen wan before and after being processed on intestinal flora and mRNA expression of uncoupling protein 2, chin, J. Exp. Tradit. Med. Formulae 22 (2016) 6–9, https://doi.org/10.13422/j.cnki.syfjx.2016140006.

[32] F. Simão, A. Matté, C. Matté, F.M. Soares, A.T. Wyse, C.A. Netto, C.G. Salbego, Resveratrol prevents oxidative stress and inhibition of Na(+)K(+)-ATPase activity induced by transient global cerebral ischemia in rats, J. Nutr. Biochem. 22 (2011) 921–928, https://doi.org/10.1016/j.jnutbio.2010.07.013.

[33] X.Y. Li, J.Y. Zhu, Y. Wu, R. Yu, Z.J. Tan, Correlation between kidney function and intestinal biological characteristics of adenine and *folium sennae* induced diarrhea model in mice, Turkish, J. Gastroenterol. 34 (2023) 4–12, https://doi.org/10.5152/tjg.2022.211010.

[34] L. Qiu, Q.J. Zhao, Z.L. Dai, N. Deng, X.J. Liu, F.L. Hu, Exploration of the mechanism of "less fire and more energy" formulation of Kidney Qi Pill based on mitochondrial energy metabolism, Lishizhen. Med. Mater. Med. Res. 30 (2019) 1850–1853.

[35] K. Martinez-Guryn, N. Hubert, K. Frazier, S. Urlass, M.W. Musch, P. Ojeda, J.F. Pierre, J. Miyoshi, T.J. Sontag, C.M. Cham, C.A. Reardon, V. Leone, E.B. Chang, Small intestine microbiota regulate host digestive and absorptive adaptive responses to dietary lipids, Cell Host Microbe 23 (2019) 458–469, https://doi.org/10.1016/j.chom.2018.03.011.

[36] M.J. Ryan, M. Schloter, G. Berg, T. Kostic, L.L. Kinkel, K. Eversole, J.A. Macklin, B. Schelkle, M. Kazou, I. Sarand, B.K. Singh, D. Fischer, E. Maguin, I. Ferrocino, N. Lima, R.S. McClure, T.C. Charles, R.S.C. de Souza, G.S. Kiran, H. L Krug, J. Taffner, H. Roume, J. Selvin, D. Smith, D. Rybakova, A. Sessitsch, Development of microbiome biobanks – challenges and opportunities, Trends Microbiol. 29 (2021) 89–92, https://doi.org/10.1016/j.tim.2020.06.009.
[37] C.X. Long, H.Q. Shao, C.Y. Luo, R. Yu, Z.J. Tan, Bacterial diversity in the intestinal mucosa of dysbiosis diarrhea mice treated with qiweibaizhu powder, Gastroenterol. Res. Pract. (2020), 9420129, https://doi.org/10.1155/2020/9420129.
[38] J. Jin, R.Y. Liu, J. Huang, Z.P. Kang, Y.B. Zhong, J. Long, H.M. Zhao, Regulation of Sishen pills, ershen pills and wuweizi powder on intestinal micro- flora imbalance in mice with colitis, Chin. Arch. Tradit. Chin. Med. (2022) 1–17.
[39] F. Chen, Y.T. Yin, H.M. Zhao, H.Y. Wang, Y.B. Zhong, J. Long, D.Y. Liu, Sishen pill treatment of DSS-induced colitis via regulating interaction with inflammatory dendritic cells and gut microbiota, Front. Physiol. 11 (2020) 801, https://doi.org/10.3389/fphys.2020.00801.
[40] X.D. Wang, C.T. Wang, X.D. Yang, Study of the function of Si shen wan to alterate the intestinal flore and to protect the intestines in mice, J. Mudanjiang. Medic. Uni. 28 (2007) 1–3, https://doi.org/10.13799/j.cnki.mdjyxyxb.2007.01.001.
[41] Y. Liu, Y. Jin, J. Li, L. Zhao, Z.T. Li, J. Xu, F.Y. Zhao, J. Feng, H.N. Chen, C.Y. Fang, R. Shilpakar, Y.W. Wei, Small bowel transit and altered gut microbiota in patients with liver cirrhosis, Front. Physiol. 9 (2018) 470, https://doi.org/10.3389/fphys.2018.00470.
[42] C.R. Li, K. Zhou, N.Q. Xiao, M.J. Peng, Z.J. Tan, The effect of qiweibaizhu powder crude polysaccharide on antibiotic-associated diarrhea mice is associated with restoring intestinal mucosal bacteria, Front. Nutr. 9 (2022), 952647, https://doi.org/10.3389/fnut.2022.952647.
[43] S.Y. Chen, Y.A. Li, B.X. Hu, L.X. Yuan, N. Liu, Y.H. Zhu, J.F. Wang, *Lactobacillus johnsonii* L531 alleviates the damage caused by Salmonella typhimurium via inhibiting TLR4 , NF- κB , and NLRP3 inflammasome Signaling pathways, 1983, Microorganisms 9 (2021), https://doi.org/10.3390/microorganisms9091983.
[44] G. Yang, E. Hong, S. Oh, E. Kim, Non-viable *Lactobacillus johnsonii* JNU3402 protects against diet-induced obesity, Foods 9 (2020) 1494, https://doi.org/10.3390/foods9101494.
[45] X.Y. Li, C.Y. Zhang, H.Y. Hui, Z.J. Tan, Effect of Gegenqinlian decoction on intestinal mucosal flora in mice with diarrhea induced by high temperature and humidity treatment, 3 Biotech 11 (2021) 83, https://doi.org/10.1007/s13205-020-02628-0.
[46] C.X. Long, Y.W. Liu, L. He, Q.Q. Tan, Z.Z. Yu, N.Q. Xiao, Z.J. Tan, Bacterial lactase genes diversity in intestinal mucosa of mice with dysbacterial diarrhea induced by antibiotics, 3 Biotech 8 (2018) 176, https://doi.org/10.1007/s13205-018-1191-5.
[47] C.X. Long, L. He, Y.F. Guo, Y.W. Liu, N.Q. Xiao, Z.J. Tan, Diversity of bacterial lactase genes in intestinal contents of mice with antibiotics-induced diarrhea, World, J. Gastroenterol. 23 (2017) 7584–7593, https://doi.org/10.3748/wjg.v23.i42.7584.
[48] K. Zhou, N. Deng, X. Yi, Y. Cai, M.J. Peng, N.Q. Xiao, Baohe pill decoction for diarrhea induced by high-fat and high-protein diet is associated with the structure of lactase-producing bacterial community, Front. Cell. Infect. Microbiol. 12 (2022), 1004845, https://doi.org/10.3389/fcimb.2022.1004845.