Koisio Technology-Produced Water Significantly Increased the Abundance of Beneficial Gut Bacterium and Decreased the Abundance of Harmful Gut Bacterium of Mice

Authors: Yinghui Men, Weihai Ying, Weihai Ying

Date: 2025-02-19T00:00:00+00:00

Abstract

Increasing evidence has indicated that gut microbiota plays crucial roles in multiple important biological processes such as energy metabolism and immunological functions. Alterations of gut microbiota also contribute significantly to the pathogenesis of a number of diseases. Our previous study has reported that Koisio technology-produced water (KW) led to significantly decreased inflammation and oxidative stress in both cell culture studies and animal studies. In this study we investigated the effects of KW drinking on the gut microbiota of mice, obtaining the following findings: First, KW drinking significantly increased the abundance of several beneficial genera of the gut bacterium including Akkermansia, Faecalibaculum, Ligilactobacillus, Lachnospiraceae and Roseburia; second, KW drinking significantly decreased the abundance of several harmful genera of the gut bacterium including Clostridioides, Escherichia-Shigella, and Enterococcus; and third, KW drinking significantly increased the abundance of Verrocomicrobiota, while it significantly decreased the abundance of Proteobacteria of the gut microbiota. Moreover, drinking of KW significantly increased the diversity and richness of the gut microbiota. Collectively, our study has obtained novel findings that KW is capable of not only increasing significantly the abundance of beneficial gut bacterium and decreasing significantly the abundance of harmful gut bacterium, but also increasing the diversity and richness of the gut microbiota of mice.

Full Text

Preamble

Koisio Technology-Produced Water Significantly Increased the Abundance of Beneficial Gut Bacteria and Decreased the Abundance of

Harmful Gut Bacteria in Mice

Yinghui Men, Weihai Ying#

School of Biomedical Engineering and Med-X Research Institute, Shanghai Jiao Tong University, Shanghai, P.R. China

Corresponding Author:

Weihai Ying, Ph.D., Professor
School of Biomedical Engineering and Med-X Research Institute, Shanghai Jiao Tong University, Shanghai 2000303, P.R. China
E-mail: weihaiy@sjtu.edu.cn

Running Title: Koisio Water Improves Gut Microbiota

Abstract

Increasing evidence indicates that gut microbiota plays crucial roles in multiple important biological processes such as energy metabolism and immunological functions, and that alterations of gut microbiota contribute significantly to the pathogenesis of numerous diseases. Our previous study reported that Koisio technology-produced water (KW) significantly decreased inflammation and oxidative stress in both cell culture and animal models. In this study, we investigated the effects of KW consumption on the gut microbiota of mice and obtained the following findings: First, KW drinking significantly increased the abundance of several beneficial bacterial genera including *Akkermansia*, *Faecalibaculum*, *Ligilactobacillus*, *Lachnospiraceae*, and *Roseburia*; second, KW drinking significantly decreased the abundance of several harmful bacterial genera including *Clostridioides*, *Escherichia-Shigella*, and *Enterococcus*; and third, KW drinking significantly increased the abundance of Verrucomicrobiota while significantly decreasing the abundance of Proteobacteria in the gut microbiota. Moreover, KW drinking significantly increased the diversity and richness of the gut microbiota. Collectively, our study obtained novel findings demonstrating that KW is capable of not only significantly increasing the abundance of beneficial gut bacteria and decreasing the abundance of harmful gut bacteria, but also enhancing the diversity and richness of the gut microbiota in mice.

Keywords: Beneficial gut bacterium; Harmful gut bacterium; Abundance; Koisio water; Mice

Introduction

A large number of studies have indicated that alterations in gut microbiota represent novel common mechanisms underlying numerous diseases, novel common biomarkers for disease diagnosis, and novel common therapeutic targets

for disease treatment [1-6]. The gut microbiota plays important roles in multiple biological processes such as nutrient digestion, host immunity, and defense against pathogenic microbial colonization [3, 6, 7]. Numerous studies have also demonstrated that alterations in gut microbiota are causative factors in multiple diseases, including metabolic diseases such as Type II diabetes, digestive system diseases such as inflammatory bowel disease, neuropsychiatric diseases such as autism, and age-associated diseases [2-5]. Therefore, identifying novel approaches that can enhance the healthy state of gut microbiota is of significant scientific and medical importance.

Our previous study suggested that Koisio technology-produced water (KW) possesses significant antioxidant capacity [8] and anti-inflammatory capacity [9]. Since inflammation and oxidative stress can produce significant effects on gut microbiota [10], we hypothesized that KW drinking may lead to changes in gut microbiota. In this study, we used a mouse model to test this hypothesis and found that KW produced significant beneficial effects on gut microbiota, significantly increasing the abundance of beneficial gut bacteria while decreasing the abundance of harmful gut bacteria in mice.

Materials and Methods

Materials

All chemicals were purchased from Sigma (St. Louis, MO, USA) except where noted. KW was produced by the technical experts of Shanghai Koisio Food Industry Co. (Shanghai, China) according to standard procedures.

Methods

Animal Model of KW Drinking Male C57BL/6Slac mice weighing 18-24 g were housed in a specific pathogen-free facility and orally administered either KW or dH_2O for 17 days. Mice were inspected daily, and both body weight and water consumption were recorded. On the 17th day, feces were collected from the mice for determination of gut microbiota composition.

Gut Microbiome 16S rRNA Gene Sequencing To elucidate the structural and functional properties of the intestinal microbiota, high-throughput sequencing was employed to analyze the V3-V4 variable region of the 16S ribosomal RNA (rRNA) gene in fecal samples. First, genomic DNA from the gut microbiota was extracted using the E.Z.N.A® soil DNA Kit (Omega Biotek, Norcross, GA, USA). The concentration and purity of the extracted DNA were quantified using a NanoDrop spectrophotometer (ThermoFisher, Waltham, MA, USA), and DNA quality was assessed by electrophoresis on a 1% agarose gel. Second, the V3–V4 regions of the 16S rRNA gene were amplified with the primer pair 338F (5 -ACTCCTACGGGAGGCAGCAG-3) and 806R (5 -GGACTACHVGGGTWTCTAAT-3) using a thermocycler PCR system (GeneAmp 9700, ABI, Foster, CA, United States).

After PCR amplification, the product was purified using the AxyPrep DNA Gel Extraction Kit (#AP-GX-250G, Axygen Biosciences, Union City, CA, USA), and amplicon quality was measured with a Quantus™ Fluorometer (Promega, Madison, WI, USA). Amplicon sequencing was conducted on the Illumina MiSeq PE300 system (Promega, San Diego, CA, USA). For data analysis, raw sequencing data were quality-filtered using fastp (version 0.21.0) and merged by FLASH, followed by clustering into operational taxonomic units (OTUs) at a 97% similarity threshold. The sequences were then aligned against the SILVA138 database for taxonomic assignment.

Statistical Analysis R (version 4.1.3) and GraphPad 10 were used to perform general statistical analysis and visualize results using packages vegan (v2.6-4), phyloseq (v1.38.0), tidyverse (v1.3.2), ggpubr (v0.5.0), ComplexHeatmap (v2.10.0), and corrplot (v0.92). Alpha diversity was estimated using the Chao1 and Shannon indices and analyzed by two-tailed Mann-Whitney test with a significance threshold set at P values less than 0.05. Principal coordinates analysis (PCoA) based on Bray-Curtis matrices with statistical significance determined by permutational multivariate analysis of variance (PERMANOVA) was conducted to assess differences in beta diversity between groups. Other data were presented as mean ± SEM and analyzed by two-tailed Student's t-test. P values less than 0.05 were considered statistically significant.

Results

1. KW Drinking Significantly Increased the Abundance of Several Beneficial Genera and Decreased the Abundance of Several Harmful Genera of Gut Bacteria in Mice

Seventeen days after mice began drinking KW, we found that KW consumption significantly increased the abundance of several beneficial bacterial genera in the mouse gut, including *Akkermansia*, *Faecalibaculum*, *Ligilactobacillus*, *Lachnospiraceae*, and *Roseburia* (Fig. 1A [Figure 1: see original paper]-1E). We also found that KW drinking significantly decreased the abundance of several harmful bacterial genera, including *Clostridioides*, *Escherichia-Shigella*, and *Enterococcus* (Figs. 2A-2C [Figure 2: see original paper]).

2. KW Drinking Significantly Changed the Composition of Several Phyla in the Gut Microbiota of Mice

Seventeen days after mice began drinking KW, we observed changes in the relative abundance of several phyla in the gut microbiota (Fig. 3 [Figure 3: see original paper]). While KW drinking did not affect the abundance of Bacteroidota (Fig. 4A [Figure 4: see original paper]), it significantly decreased the abundance of Firmicutes (Fig. 4B). Notably, KW drinking led to a significant decrease in the abundance of Proteobacteria (Fig. 4C) while significantly increasing the abundance of Verrucomicrobiota (Fig. 4D).

3. KW Drinking Significantly Increased the Diversity and Richness of the Gut Microbiota in Mice

The Venn diagram illustrates that the KW group exhibited a higher number of unique OTUs compared to the control group (Fig. 5A [Figure 5: see original paper]), indicating a notable increase in species richness. This finding was further supported by our alpha diversity analysis: the Chao1 index indicated a marked increase in species richness (Fig. 5B), and the Shannon index indicated a significant increase in microbial diversity and evenness in the KW group (Fig. 5C). Moreover, beta diversity analysis using principal coordinates analysis (PCoA) showed distinct clustering of microbial communities between the two groups (Fig. 5D), suggesting that KW drinking induced a substantial shift in the composition of the gut microbiota.

Discussion

Our study obtained the following novel findings: First, KW drinking significantly increased the abundance of several beneficial bacterial genera in mice, including *Akkermansia*, *Faecalibaculum*, *Ligilactobacillus*, *Lachnospiraceae*, and *Roseburia*. Second, KW drinking significantly decreased the abundance of several harmful bacterial genera, including *Clostridioides*, *Escherichia-Shigella*, and *Enterococcus*. Third, KW drinking significantly increased the abundance of Verrucomicrobiota while significantly decreasing the abundance of Proteobacteria in the gut microbiota. Fourth, KW drinking significantly increased the diversity and richness of the gut microbiota in mice.

Our study demonstrated that KW drinking led to significant increases in the abundance of several beneficial bacterial genera, including *Akkermansia muciniphila*, *Faecalibacterium*, *Ligilactobacillus*, *Bacteroides*, and *Roseburia*. Numerous studies have indicated that *Akkermansia muciniphila* (A. muciniphila) is a beneficial gut bacterium that produces multiple beneficial biological effects, including decreased inflammation, improved glucose metabolism, and reduced body fat [11]. Healthy individuals exhibit higher abundance of A. muciniphila in the gut compared to individuals with metabolic disorders [11].

Faecalibacterium is one of the critical bacterial types in the human gut that possesses multiple beneficial biological functions, such as anti-inflammatory activity and production of n-butyric acid. Both experimental and epidemiological data have indicated the great potential of *Faecalibacterium* as a promising probiotic or live biotherapeutic product [12]. First, patients with gastrointestinal diseases, depression, and dermatitis show low abundance of *Faecalibacterium* in the gut, and second, low levels of *Faecalibacterium* are associated with inflammatory conditions.

Increasing evidence has also indicated that *Ligilactobacillus murinus*, a member of the *Ligilactobacillus* genus, plays beneficial roles in intestinal metabolism and host immune activities [13]. There is also a close correlation between the abundance of *Ligilactobacillus murinus* and intestinal health, suggesting its sig-

nificant potential as a probiotic [13]. Several species of *Bacteroides* in the gut belong to dominant beneficial bacteria that provide nutrition and vitamins to the host and other intestinal microbial residents by metabolizing polysaccharides and oligosaccharides [14]. The genus *Roseburia* plays beneficial biological roles by metabolizing dietary components to produce butyrate [15]. Butyrate serves several important biological functions [16]: it acts as a link between the intestinal microbiome and epithelium, providing fuel for epithelial cells; it regulates epithelial inflammation through production of anti-inflammatory cytokines; and it can produce histone modifications and altered transcriptional activation in epithelial cells, halting cell cycle progression and producing protective effects against colonic carcinogenesis.

Our study also showed that KW drinking led to significant decreases in the abundance of three harmful bacterial genera, including *Clostridioides*, *Escherichia-Shigella*, and *Enterococcus*. *Clostridioides difficile* (C. difficile) is the etiological agent of C. difficile infection (CDI), an antibiotic-associated diarrhea that can be fatal if untreated [17]. C. difficile has become an 'Urgent Threat' to U.S. healthcare, with an annual CDI burden of approximately 220,000 cases and 13,000 deaths [17]. *Shigella* belongs to harmful gut microbiota that causes bacillary dysentery in humans [18], characterized by invasion and inflammatory destruction of the human colonic epithelium [18]. The genus *Enterococcus* is a causative agent of healthcare-associated infections [19], with the majority of enterococcal infections caused by *Enterococcus faecalis* and *Enterococcus faecium*, both of which exhibit intrinsic resistance to common antibiotics [19].

Our study found that KW drinking led to a significantly decreased abundance of Proteobacteria while significantly increasing the abundance of Verrucomicrobiota. These observations are consistent with our findings stated above: since multiple genera of harmful gut bacteria belong to the phylum Proteobacteria, our finding regarding the KW-induced decrease in Proteobacteria abundance is consistent with our findings regarding KW-induced decreases in several harmful bacterial genera. Similarly, since multiple genera of beneficial gut bacteria belong to the phylum Verrucomicrobiota, our finding regarding the KW-induced increase in Verrucomicrobiota abundance is consistent with our findings regarding KW-induced increases in several beneficial bacterial genera.

Ecological theory predicts that species-rich communities are less susceptible to invasion, suggesting that higher microbial richness and diversity enhance the environmental resilience of gut microbiota. Conversely, low microbial richness and diversity correlate with obesity [20, 21], inflammatory bowel disease [22], and C. difficile-associated disease [23]. Our study found that KW drinking significantly increases the richness and diversity of gut microbiota, suggesting that KW can produce beneficial effects by enhancing microbial richness and diversity.

It is of great interest to investigate the mechanisms underlying the effects of KW drinking on gut microbiota in mice. Our previous cell culture and animal studies reported that KW possesses significant antioxidant capacity [8] and anti-

inflammatory capacity [9]. Since inflammation and oxidative stress can produce significant effects on gut microbiota [10], it is warranted to test our hypothesis that KW may affect gut microbiota through its effects on inflammatory processes and oxidative stress in the mouse gut.

References

1. Ying, W., Wang, Y., Zhou, B, China-Style Health Industry: Moving Forward Toward Future. Shanghai Science & Technology Publishing House. 2024.
2. Gurung, M., et al., Role of gut microbiota in type 2 diabetes pathophysiology. EBioMedicine, 2020. 51: p. 102590.
3. Nishida, A., et al., Gut microbiota in the pathogenesis of inflammatory bowel disease. Clin J Gastroenterol, 2018. 11(1): p. 1-10.
4. Pickard, J.M., et al., Gut microbiota: Role in pathogen colonization, immune responses, and inflammatory disease. Immunol Rev, 2017. 279(1): p. 70-89.
5. Ling, Z., et al., Gut microbiota and aging. Crit Rev Food Sci Nutr, 2022. 62(13): p. 3509-3534.
6. Álvarez, J., et al., Gut microbes and health. Gastroenterol Hepatol, 2021. 44(7): p. 519-535.
7. Adak, A. and M.R. Khan, An insight into gut microbiota and its functionalities. Cell Mol Life Sci, 2019. 76(3): p. 473-493.
8. Men, Y., Ying, W, Koisio technology-produced water significantly decreases H2O2-produced oxidative stress in both cell culture studies and animal model studies. Chinaxiv: 202502.00063v1, 2025.
9. Men, Y., Zhang, M., Zhang, Y., Ying, W, Koisio technology-produced water significantly decreased inflammation and multiple injuries in mouse model of Dextran Sulfate Sodium Salt-induced acute colon inflammation. Chinaxiv: 202212.00038, 2022.
10. Randeni, N., M. Bordiga, and B. Xu, A Comprehensive Review of the Triangular Relationship among Diet-Gut Microbiota-Inflammation. Int J Mol Sci, 2024. 25(17).
11. Aja, E., et al., Health Effects and Therapeutic Potential of the Gut Microbe Akkermansia muciniphila. Nutrients, 2025. 17(3).
12. Martín, R., et al., Faecalibacterium: a bacterial genus with promising human health applications. FEMS Microbiol Rev, 2023. 47(4).
13. Chuandong, Z., et al., Distribution and roles of Ligilactobacillus murinus in hosts. Microbiol Res, 2024. 282: p. 127648.

14. Zafar, H. and M.H. Saier, Jr., Gut Bacteroides species in health and disease. Gut Microbes, 2021. 13(1): p. 1-20.

15. Tamanai-Shacoori, Z., et al., Roseburia spp.: a marker of health? Future Microbiol, 2017. 12: p. 157-170.

16. Salvi, P.S. and R.A. Cowles, Butyrate and the Intestinal Epithelium: Modulation of Proliferation and Inflammation in Homeostasis and Disease. Cells, 2021. 10(7).

17. Anwar, F. and G. Vedantam, Surface-displayed glycopolymers of Clostridioides difficile. Curr Opin Microbiol, 2022. 66: p. 86-91.

18. Belotserkovsky, I. and P.J. Sansonetti, Shigella and Enteroinvasive Escherichia Coli. Curr Top Microbiol Immunol, 2018. 416: p. 1-26.

19. García-Solache, M. and L.B. Rice, The Enterococcus: a Model of Adaptability to Its Environment. Clin Microbiol Rev, 2019. 32(2).

20. Turnbaugh, P.J., et al., Diet-Induced Obesity Is Linked to Marked but Reversible Alterations in the Mouse Distal Gut Microbiome. Cell Host & Microbe, 2008. 3(4): p. 213-223.

21. Vangay, P., et al., US Immigration Westernizes the Human Gut Microbiome. Cell, 2018. 175(4): p. 962-972.e10.

22. Willing, B.P., et al., A Pyrosequencing Study in Twins Shows That Gastrointestinal Microbial Profiles Vary With Inflammatory Bowel Disease Phenotypes. Gastroenterology, 2010. 139(6): p. 1844-1854.e1.

23. Chang, J.Y., et al., Decreased Diversity of the Fecal Microbiome in Recurrent Clostridium difficile—Associated Diarrhea. The Journal of Infectious Diseases, 2008. 197(3): p. 435-438.

Figure Legends

Figure 1. KW drinking significantly increased the abundance of several beneficial genera of gut bacteria in mice. Seventeen days after mice began drinking KW, we found that KW consumption significantly increased the abundance of several beneficial bacterial genera in the mouse gut, including *Akkermansia* (A), *Faecalibaculum* (B), *Ligilactobacillus* (C), *Lachnospiraceae* (D), and *Roseburia* (E). Data are presented as mean ± SEM. Statistical significance was determined using two-tailed Student' s t-test. #, $P < 0.05$; ##, $P < 0.01$. $N = 6$.

Figure 2 [Figure 2: see original paper]. KW drinking significantly decreased the abundance of several harmful genera of gut bacteria in mice. Seventeen days after mice began drinking KW, we found that KW consumption significantly decreased the abundance of several harmful bacterial genera in the mouse gut, including *Clostridioides* (A), *Escherichia-Shigella* (B), and *Enterococcus* (C). Data are presented as mean ± SEM. Statistical significance was determined using two-tailed Student' s t-test. ###, $P < 0.001$. $N = 6$.

Figure 3 [Figure 3: see original paper]. KW drinking changed the distribution of several phyla in the gut microbiota of mice. Seventeen days after mice began drinking KW, the relative distribution of several phyla in the mouse gut microbiota was determined. The dominant phyla include Bacteroidota (blue), Firmicutes (orange), Verrucomicrobiota (green), Proteobacteria (red), Cyanobacteria (purple), and Others (brown). N = 6.

Figure 4 [Figure 4: see original paper]. KW drinking significantly changed the abundance of several phyla in the gut microbiota of mice. Seventeen days after mice began drinking KW, the abundance of several phyla in the mouse gut microbiota was determined. While KW drinking did not affect the abundance of Bacteroidota (A), it significantly decreased the abundance of Firmicutes (B). KW drinking also significantly decreased the abundance of Proteobacteria (C) while significantly increasing the abundance of Verrucomicrobiota (D) in the mouse gut. #, $P < 0.05$; ##, $P < 0.01$. N = 6.

Figure 5 [Figure 5: see original paper]. KW drinking significantly increased the diversity and richness of the gut microbiota in mice. (A) The Venn diagram illustrates that the KW group exhibited a higher number of unique OTUs compared to the control group, indicating a notable increase in species richness. (B) Alpha diversity analysis using the Chao1 index showed a marked increase in species richness. (C) Alpha diversity analysis using the Shannon index showed a significant increase in microbial diversity and evenness in the KW group. (D) Beta diversity analysis using principal coordinates analysis (PCoA) showed distinct clustering of microbial communities between the two groups, suggesting that KW drinking induced a substantial shift in the composition of the gut microbiota. **, $P < 0.01$. N = 6.

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