



Research article

The effects of high-fat foods on gut microbiota and small molecule intestinal gases: release kinetics and distribution *in vitro* colon modelZhi-tao Li^{a,b,c}, Jia-wei Wang^a, Xing-hai Hu^a, Li Zhu^a, Yun Jiang^a, Min-jie Gao^{a,**}, Xiao-bei Zhan^{a,*}^a Key Laboratory of Carbohydrate Chemistry and Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, Wuxi 214122, China^b State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, 214122, China^c School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, 214122, China

HIGHLIGHTS

- High-fat foods (HFF) can alter the gut microbiota and its metabolites.
- HFF stimulate H₂S and volatile organic compound production in the colon.
- Specific intestinal gases can be used as disease markers.

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ABSTRACT

Profiling intestinal gases and their responses to dietary changes can reveal the products and functions of the gut microbiota and their influence on human health. High-fat foods (HFF) can alter the gut microbiota and its metabolites, posing a potential health risk. However, little is known about the effects of HFF on intestinal gas distribution. Therefore, in this study, we used human fecal microorganisms as strains, an *in vitro* three-chamber colon model and an intestinal gas array sensor as tools. We performed *in vitro* fermentation using HFF as the fermentation substrate to reveal the effects of HFF on the kinetics of intestinal gas production and changes in the gut microbiota and its metabolites. We found that dietary fatty acids stimulated the production of H₂S and volatile organic compounds in the colon, promoted Firmicutes abundance, and decreased Bacteroidetes abundance. These results highlight the potential role of HFF in altering the gut microbiota and intestinal gas, which can lead to health hazards.

1. Introduction

High-fat foods (HFF) represent a public health concern and pose a significant health challenge [28]. Consumption of HFF saturates the ability of the small intestine to emulsify and absorb all dietary lipids. Hence, a considerable fraction of lipids is not absorbed and end up reaching the colon. Much less is known about the microbial degradation of dietary fat in the human gut. To date, only a few studies have investigated the influence of dietary fats on the human gut microbiota, and almost none have focused on intestinal gas production [1]. Intestinal gas is a disease biomarker with high diagnostic/detection value and is clinically important in the

pathogenesis of gut diseases [10], especially irritable bowel syndrome (IBS) [16, 25], inflammatory bowel disease (IBD) [12, 17], and colorectal cancer (CRC) [2, 4].

Despite the recognition of the detrimental gut microbiota-mediated effects of HFF on human health, there is a significant gap in our understanding regarding the populations of the microbiota that are primarily responsible for lipid degradation, and the specific consequences of HFF consumption on the structure and functions of the microbiota, including gas production [1]. To a large extent, the lack of such information can be related to the extreme difficulty of providing a fat-only diet and collecting intestinal gas from human volunteers or

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rodent animals. In this study, we used a newly designed multivessel *in vitro* bionic colon model system and gas-profiling technology, with the specific goal of studying the potential impact of the gut microbiota on the utilization of HFF for colonization, growth, and gas production.

2. Materials and methods

2.1. Dietary medium

With reference to the nutrient intake of males in the Dietary Nutrient Reference Intakes for Chinese Residents [24], two chyme media that mimic the digestion of the small intestine were configured to provide nutrients to the gut microbiota in the proximal colon, as shown in Table 1. The BM (eliminating lipids) contained carbohydrates, and protein in proportions that matched the expected distribution of dietary nutrients in the colon of adult males. The HFF (eliminating carbohydrates) had protein content and a high level of dietary fatty acids. In addition, during actual human digestion, components such as secreted pancreatic enzymes, bile salts, and shed mucin from the small intestine are transported to the colon along with chyme, so mucin, pancreatic enzymes, and bile salts (all of porcine origin) were added to the nutrient composition of the medium. Cysteine and sodium sulfate were added to investigate the differences in H₂S production and sulfur-containing substrates metabolized by the gut microbiota.

2.2. Preparation of fecal microbiota inocula

Before beginning the experiment, the experimental protocol was established according to the ethical guidelines of the Helsinki Declaration.

Table 1. Medium composition.

Medium component (g/l)	Medium ^a		
	BM	HFF	PYEM
Carbohydrates			
Arabinogalactan	1.8		
Cellobiose	0.9		
Fructose	0.8		
Glucose	0.7		
Guar gum	1.1		
Inulin	1.1		
Pectin	1.8		
Starch	4.4		
Xylan	0.9		
Proteins			
Casein	2.0	2.0	2.0
Peptone	1.9	1.9	1.9
Lipids			
Capric acid (C _{10:0})		0.5	
Palmitic acid (C _{16:0})		1.6	
Stearic acid (C _{18:0})		0.8	
Oleic acid (C _{18:1})		1.8	
Linoleic acid (C _{18:2})		1.4	
Mucin	4.0	4.0	4.0
Yeast extract	3.0	3.0	3.0
Sodium chloride	5.0	5.0	5.0
Disodium hydrogen phosphate	3.5	3.5	3.5
Potassium dihydrogen phosphate	3.5	3.5	3.5
Trypsin	2.5	2.5	2.5
Bile salts	1.0	1.0	1.0

^a BM, basal medium; HFF, high-fat foods medium; PYEM, peptone-yeast extract medium.

Three healthy donors (aged between 25 and 30 years) were recruited, they had no gastrointestinal diseases, had not taken antibiotics or probiotics for at least 6 months. Informed consent was obtained from all participants, as required by the Human Ethics Committee of Affiliated Hospital of Jiangnan University (approval No. S2021-12-15) on the Use of Humans as Experimental Subjects. Fresh stool samples were provided on the day of the fermentation test, and all stool samples were mixed and homogenized under anaerobic conditions, filtered through sterile gauze, and frozen with the addition of 10% phosphate buffer.

2.3. In vitro bionic colon model system

The system used was based on a previously published multi-chamber bionic colonic model system (shown in Figure 1A) consisting of three consecutive colonic bioreactors. The three colonic bioreactors simulated the environmental conditions of different regions of the colon containing human intestinal microorganisms; reactor 1 simulated the proximal ascending colon, reactor 2 simulated the transverse colon, and reactor 3 simulated the distal descending colon. Three sets of bionic colonic bioreactor systems were operated together to reduce experimental errors. Baseline control group: Each reactor was spiked with 200 mL of BM, inoculated with 5% fecal slurry, and incubated for 12 h to allow stable colonization of the gut microbiota in the reactor. A flow of 50 mL/d of BM was added to the simulated proximal ascending colon reactor 1 and fermented for 48 h with the purpose of replenishing the nutrients in the reactor and maintaining a stable gut microbiota. Experimental control group: Each reactor was spiked with 200 mL of BM, inoculated with 5% fecal slurry, and incubated for 12 h to allow stable colonization of the gut microbiota in the reactor. A flow of 50 mL/d of BM was added to the simulated proximal ascending colon reactor 1 and fermented for 24 h with the purpose of replenishing the nutrients in the reactor and maintaining a stable gut microbiota. At the end of 24 h, the BM of the three-compartment colonic model was replaced with the HFF presented in Table 1, and the colonic model systems were continued to run for 24 h in the same way. In addition, we conducted a blank control experiment in which the BM of the systems was replaced with peptone-yeast extract medium (PYEM) at the end of 24 h and continued to run for an additional 24 h to exclude the effect of the nutrient content of yeast paste. The anaerobic environment in the reactor was maintained by charging high-purity nitrogen (an inert gas that does not affect the newly generated gas) during the fermentation process. The temperature in the model was set at 37 °C; the peristaltic frequency was 4 contractions per min, the peristaltic pump was controlled; the pH feedback controller was used to adjust the pH in the model; and a disposable sterile syringe was used to take samples at regular intervals.

2.4. Real-time gut gases detection system

Gases such as N₂, CO₂, H₂, CH₄, H₂S, NO, and VOCs produced in the bionic colon model system were monitored in real time by gas sensors using the electrochemical principle. The gas sensor was connected to a collection bottle. The bottle sealing device had an inlet and an exhaust port, as shown in Figure 1A. The gas inlet of the device was connected to the bionic colonic model, and the size of the airflow was adjusted using a peristaltic pump. The total gas yield was measured every 4 h. The data generated were converted to unit volumes using the ideal gas Eq. (1) of the state [33]:

$$pV = nRT \quad (1)$$

where p is the pressure of the ideal gas, V is the volume of the ideal gas, n is the amount of gas substance, T is the thermodynamic temperature of the ideal gas, and R is the ideal gas constant. The gas yields for the different gas species were calculated by multiplying the gas concentration by the volume at each time point, and the gas production rates were

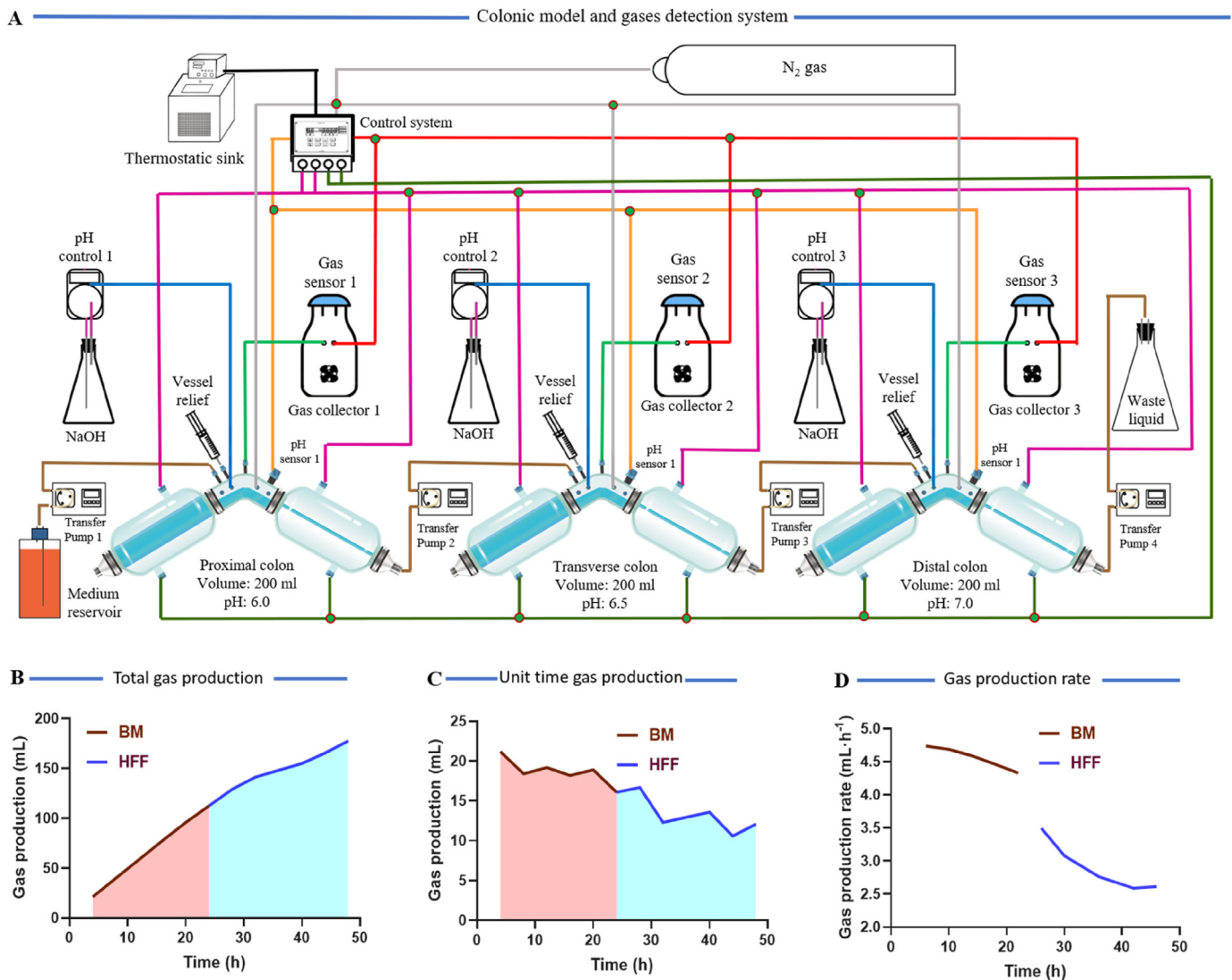


Figure 1. Three-compartment colonic model with a real-time gut gases detection system for the measurement of total gas production. (A) Schematic representation of the three-compartment colonic model and real-time gut gases detection system; (B) Total gas production of three-compartment colonic model; (C) Unit time gas production; (D) Gas production rate. BM, basal medium; HFF, high-fat foods medium.

calculated using the derivative of the cumulative gas production or concentration Eq. (2) as follows [33]:

$$v = \frac{d}{dt}y(t) \quad (2)$$

2.5. Total gas production

Gas production was adjusted for *in vitro* bionic colon model total headspace volume, converted from pressure (psi) into volume (mL) using the 'ideal' gas and Avogadro's laws, and gas production of different gas species was calculated by multiplying the gas concentration at each timepoint with the headspace volume. Cumulative gas production at the end of the 48-hour incubation period was used to assess the total extent of fermentation. Variations in total gas production between individual colon model were minimal over the 48-hour experimental period, demonstrated in separate experiments involving triplicate fermentations evaluating.

2.6. 16S rRNA amplicon-based sequencing and data analysis

The simulate fecal fermentation samples were loaded into 2 mL sterilized centrifuge tubes and the microbial nucleic acids were

extracted with phenol-chloroform-isoamyl alcohol. Bacterial universal primers 515F and 907R were selected to amplify the 16S rRNA gene V3 to V4 region of bacteria. The amount of DNA extracted was measured by the QIAamp® DNA Stool Mini Kit (QIAGEN, Germany), and sequence analysis was performed on the Illumina MiSeq platform according to the established procedure. Operational taxonomic units (OTU) clustering analysis was performed on the screened sequences using UPARSE software, and the Chao1 index, an index of colony richness, was evaluated using Mothur software, and the Shannon and Simpson indices, an index of colony diversity, were used to compare the groups at the phylum level, genus level, species level and OTU level. The differences in abundance between groups at the phylum level, genus level, species level and OTU level were compared, and the core OTU of each group was analyzed by Venn diagram.

2.7. Statistical analysis

Statistical analyses with R statistical software (version 4.0.2; R Foundation for Statistical Computing), and plot with Graphpad Prism (Version 8.4.3; Graphpad Software), data are presented as mean (standard error of the mean [SEM]).

3. Results

3.1. High-fat diet inhibits gas production in the colon in vitro

The colonic model used in this study simulated the human gastrointestinal tract. We designed two chyme media (basal medium (BM) and high-fat diet medium (HFF)) that mimic digestion in the small intestine to provide nutrients to the gut microbiota in the colon in proportions that match previous reports of the human colon based on the Dietary

Nutrient Reference Intakes for the population of adult men in China (Table 1) [24].

Based on a previously published model [13, 14, 15], we constructed a three-compartment colonic model system. The system design is illustrated in Figure 1A. Each compartment of the colonic model system was inoculated with an aliquot of fecal microorganisms from healthy humans and maintained a stable growth of the gut microbiota. The compartments of the model system were connected to a multi-channel gas sensor that detects gas production in real time to construct a kinetic model of intestinal gas production.

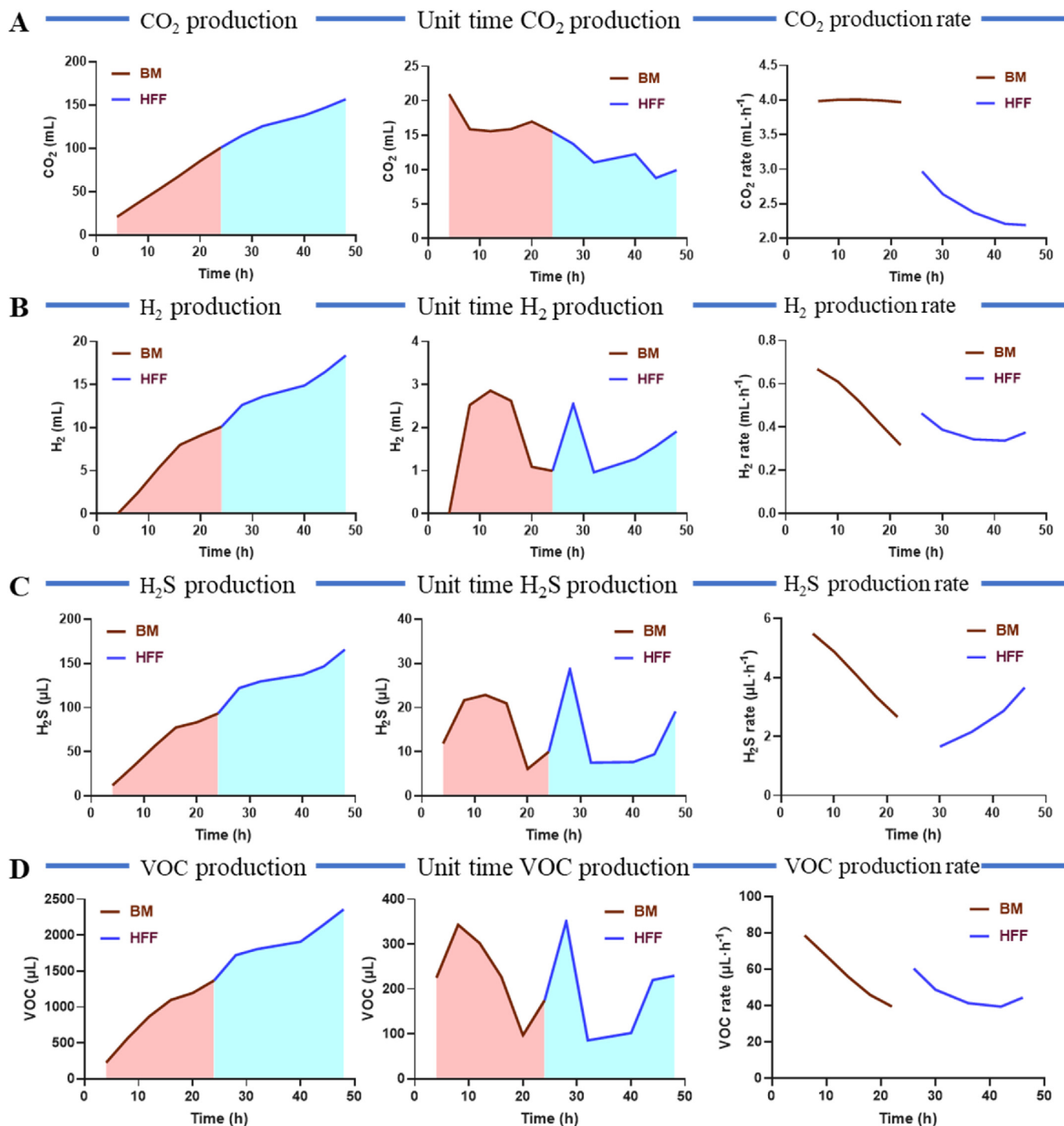


Figure 2. Production kinetics of CO_2 , H_2 , H_2S and volatile organic compounds (VOC) in basal medium (BM) and high-fat foods medium (HFF). (A) CO_2 production rate; (B) H_2 production rate; (C) H_2S production rate; (D) VOC production rate.

We found that total gas production was steadily elevated using BM growth, reaching 112.2 mL after 24 h. After the addition of HFF, total gas production slowed down, reaching 177.3 mL after 48 h (Figure 1B). The gas production per unit of time gradually decreased over time (Figure 1C). First-order derivative analysis of gas production and time showed that the rate of gas production decreased significantly from 24 to 48 h (Figure 1D).

3.2. High-fat diet promotes H₂S and volatile organic compound (VOC) production in the colon in vitro

Gas composition was analyzed, and the results revealed that the gas was mainly composed of CO₂, H₂, H₂S, and VOCs. However, the rate of CO₂ and H₂ production slowed down after the addition of HFF compared to BM (Figure 2A and B), and the rate of H₂S and VOC production increased significantly (Figure 2C and D). We used fecal samples from CH₄ non-producers [33], and hence no CH₄ was produced.

3.3. High-fat diet alters the distribution of gas-producing microbiota in the colon in vitro

We compared the changes in microbiota composition between the BM and HFF groups. As a result, the Venn diagram displayed 193 unique operational taxonomic units (OTUs) in the BM group and 145 unique

OTUs in the HFF group. A total of 305 OTUs were shared by both groups (Figure 3A). The BM and HFF gut microbial community structures were significantly different (Figure 3B). At the phylum level, compared with the BM group, the HFF group was characterized by higher Firmicutes levels and a significantly higher Firmicutes/Bacteroidetes ratio (Figure 3C). Linear discriminant analysis (LDA) [22] and distribution diagram analysis (LDA score >3.5) showed a clear alteration of the microbiota in the HFF group, characterized by higher abundance of *Megamonas* and Negativicutes associated with H₂S production, and higher *Selenomonadaceae* and *Roseisolibacter* levels associated with VOC production (Figure 3D). However, *Bacteroides* and *Roseburia* levels (associated with H₂ production) and *Dehalococcoidia* levels (associated with the CO₂ cycle) were significantly decreased in the HFF group (Figure 3E).

4. Discussion

CO₂ is the main gas produced by chemical action of the stomach and by bacterial fermentation of carbohydrates in the distal small intestine and colon. It is an inert gas and therefore may have no relevant influence other than mechanical stimulation [3]. H₂ is mainly produced by intestinal fermentation of *Roseburia*, *Ruminococcus gnavus*, *Clostridium*, *Prazmowski*, and *Bacteroides* fermentation. It is the main gas marker of carbohydrate fermentation and can be used as a diagnostic test for carbohydrate malabsorption or intestinal bacterial overgrowth [5, 7]. H₂S is

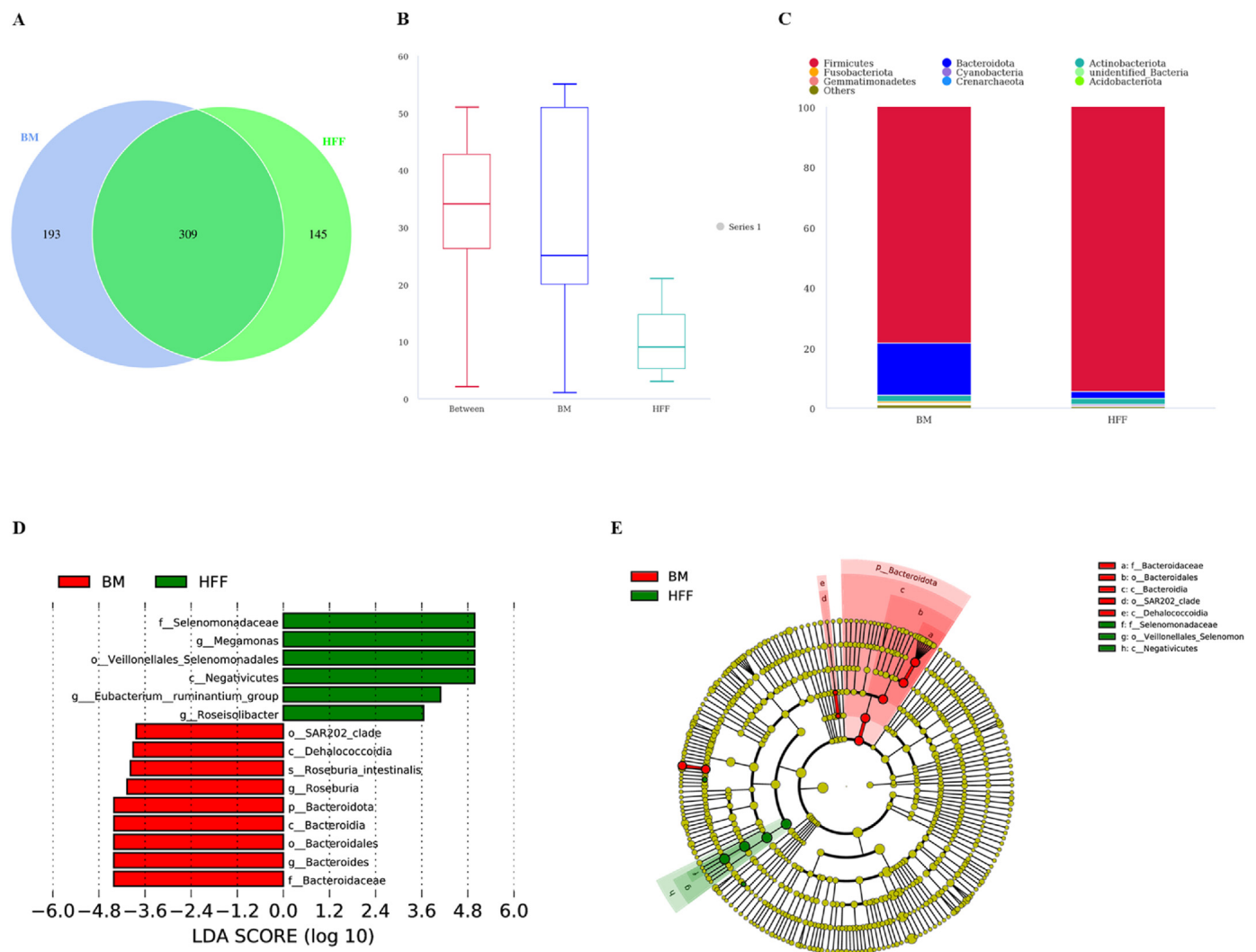


Figure 3. Shift of gut microbiota in basal medium (BM) and high-fat diet medium (HFF) according to the 16S rRNA data. (A) Operational taxonomic units (OTUs); (B) Analysis of similarities (ANOSIM); (C) Phylum level; (D) Linear discriminant analysis (LDA); (E) Distribution diagram analysis.

produced by sulfate-reducing bacilli or bacteria through the fermentation of sulfur-containing amino acids and may have both beneficial and toxic effects. On the one hand, H₂S as a gaseous transmitter may contribute to the treatment of colitis by reducing malnutrition and aiding in the reconstruction of the mucus layer. On the other hand, H₂S is a toxic gas and is considered harmful at high concentrations [27]. The most common VOCs in the intestine include ammonia, branched-chain amines, branched-chain fatty acids, indoles, phenols, and volatile sulfur-containing compounds, which are mainly metabolized by intestinal microorganisms. VOCs are strongly correlated with the development of various intestinal diseases (especially functional disorders, cancer, and inflammation) [4], making them valuable for assessing pathological processes and predicting therapeutic response in IBS.

Based on the HFF, gut microbes, and gas distribution changes described above, various potential applications are worth discussing. Profiling intestinal gases enables the functional assessment of the gut microbiome. For example, intestinal gas composition can influence gut physiology and generate abdominal symptoms in patients with gut disorders such as IBS, IBD, and CRC [11, 19, 20]. This study found that the main metabolic gases in the colon of HFF are CO₂ and H₂, which can stimulate an increase in H₂S and VOCs and may increase the probability of IBD, IBS, and CRC. HFF can affect host health through increased lipopolysaccharide (LPS) exposure, and a strong correlation between LPS levels and disease severity has been observed in patients with IBD. However, the detailed mechanisms by which elevated LPS exposure is associated with disease remain to be fully elucidated. Based on the studies in mice, it may be multifactorial, such as increased abundance of gram-negative bacilli [21], decreased inhibition of LPS-induced inflammation by bacteria such as *Bifidobacterium* [8].

In our study, we observed that HFF was associated with significant changes in the microbiome, particularly in the distribution of gas-producing microbiomes, including *Megamonas* [23], *Negativicutes* [29], *Selenomonadaceae* [26], *Roseisolibacter* [18], *Bacteroides* [34], *Roseburia* [6], and *Dehalococcoidia* [32], which may be closely associated with the occurrence of various gut diseases, particularly dysfunction, cancer, and inflammation. Specific types of fat have a significant impact on gut microbiota and host health outcomes. And consumption of walnuts rich in unsaturated fat enriches the gut of healthy adults with *Bacillus faecalis*, *Roseisolibacter* and *Fusobacterium*, thereby reducing microbial-derived pro-inflammatory secondary bile acids [9, 30, 31]. Agans et al. [1] found that the growth of the human gut microbiota may be sustained on a fat-only medium, but the production of short-chain fatty acids and antioxidants is severely reduced in such conditions, predicting a significant increase in prevalence. 40% decrease in the overall total gas and 70% increase in VOCs by the HFF in comparison to BM. Interestingly, our findings provide mechanistic evidence for the increased prevalence of specific gases in the gut of humans and animals fed HFF.

The above findings challenge the comprehensive classification of HFF as pro-inflammatory diets. However, gastric and small intestinal digestion were not simulated in this study, and there may be some errors when compared to *in vivo*, but highlight the need for further research on how specific dietary fats regulate intestinal gas or host-gut gas interactions. Analysis of specific types of fats and their effects on intestinal gases is key to the precise design of the microbiome using dietary fats. In the future, gas analysis will likely play an important role in the prevention, diagnosis, and monitoring gut diseases. The results of this study have important implications for the establishment of a database on the correspondence between diet, intestinal gas, and disease, and provide a reference for the use of intestinal gas profiling techniques for individualized diet planning.

5. Conclusion

This study aimed to investigate the interaction between HFF and intestinal gas, and to model the kinetics of intestinal gas production in the colon using an *in vitro* model. The results indicate that HFF stimulates the

production of H₂S and VOCs in the colon, promoting the proliferation of *Firmicutes* and reducing the abundance of *Bacteroidetes*. It could be concluded that HFF plays a role in altering the gut microbiota and intestinal gas, which can lead to health problems. It is believed that this study makes a significant contribution to the literature because there is a lack of information on the effects of HFF on intestinal gas distribution.

Declarations

Author contribution statement

Zhi-tao Li: Conceived and designed the experiments; Analyzed and interpreted the data; wrote the paper.

Jia-wei Wang: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Xing-hai Hu: Analyzed and interpreted the data; Wrote the paper.

Li Zhu; Yun Jiang: Performed the experiments; Analyzed and interpreted the data.

Min-jie Gao: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Xiao-bei Zhan: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at <https://doi.org/10.1016/j.heliyon.2022.e10911>.

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