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Article

Characteristics of Dietary Fiber-Induced Effects on the Gut Microbiota and Metabolites Associated with a High-Meat Diet in Children

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Abstract: The gut microbiota plays a crucial role in childhood obesity, and diet is a dominant driver. The effects of fructo-oligosaccharides (FOS), as a dietary fiber, on the composition and metabolism of gut microbiota in healthy children were investigated by an in vitro fermentation system with a reformative YCFA (yeast extract, casein hydrolysate, fatty acids) medium (rich in tryptic hydrolysates of meat). 16S rRNA sequencing technology was utilized to analyze the varieties of gut microbiota. Short-chain fatty acids (SCFAs) and gases were measured by gas chromatography. The Majorbio Cloud Platform and MetOrigin, as interactive cloud servers, perform microbiota analysis, metabolic pathway enrichment analysis, statistical correlations, and biological relationships using network visualization. We found that FOS significantly regulated the composition and metabolism of the gut microbiota. The co-metabolism network showed that three metabolites were related to six differential bacteria and eight metabolism pathways. These findings suggest that dietary fiber could regulate the composition of the gut microbiota and its metabolites in a favorable way; however, when dietary fiber participates in precision nutrition formulas, it may be relevant to precision medicine and obesity, and may help identify windows of opportunity for dietary intervention for childhood obesity.

Keywords: dietary fiber; childhood obesity; gut microbiota; metabolite; high-meat diet

1. Introduction

Obesity has become a general health issue in children in recent decades. Obesity is a higher risk factor in the process of type 2 diabetes, cardiovascular diseases, and colon cancer [1]. Childhood obesity tracks to adolescence and adulthood [2]; thus, most adolescents with obesity may have a higher risk of metabolic disorders such as dyslipidemia, insulin resistance, and hypertension in adult life [3,4].

Excessive meat intake and transformation to lipid amassing in the body are the primary drivers of obesity. In this way, a reasonable diet is a vital aspect of avoiding obesity. Dietary varieties, including high fat, high protein, and other undesirable dietary examples [5,6], are significant obesity-

causing factors except for genetic background, exercise propensities, mental status, and sleep [7]. An investigation revealed that from youth to pre-adulthood, dietary quality reductions were essential, particularly decreases in organic products, vegetable, and complete dairy consumption, followed by expanding meat consumption [8]. Mozaffarian et al. found that weight gain is positively connected with the intake of potato chips, sweet refreshments, and red meats and is conversely connected with the intake of vegetables, natural products, nuts, and yogurt [9]. In the past three decades, the progress from a customary low meat, high dietary fiber to a high meat and low dietary fiber has increased the risk of obesity in China [10]. Accordingly, further examinations of the dietary patterns influencing children's health are urgently needed.

Accumulating evidence suggests that a dysbiotic gut microbiota may contribute to diet-related obesity [11,12]. Germ-free mice were demonstrated to be impervious to high-sugar, high-fat, "Western" diet-induced obesity [13]. Transplantation of gut microbiota from corpulent mice or people expanded fat stores in germ-free mouse beneficiaries [14,15]. The advancement of adiposity and insulin resistance in mice could be fundamentally decreased by lessening the gut microbiota with broad-spectrum antibiotics [16]. Transplantation of gut microbiota from healthy benefactors further developed insulin resistance in the initial one and a half months in obese human volunteers [17]. Dietary fiber has been shown to diminish endotoxin markers and improve beneficial *Bifidobacteria* in the gut of obese volunteers, prompting diminished endotoxin in the bloodstream and critical easing of inflammation, adiposity and insulin resistance [18,19]. Thus, undeniable proof suggests that the gut microbiota is a vital contributing component in the improvement of diet-related obesity.

Having this as a main priority, the expanded interest in focusing on the gut microbiota for the treatment and counteraction of obesity is understandable. One of the significant impacts on the microbial signatures of people is diet [20], and one method for changing the microbiota is through an increase in dietary fiber intake [21]. As indicated by the United States Food and Drug Administration (FDA), dietary fiber, which is a carbohydrate part of an edible plant that is unable to be digested and absorbed, such as fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), and resistant starch, has been shown to beneficially impact the composition of gut microbiota. In this way, dietary fiber cannot be digested by the host but can be matured by gut microbes in the distal digestive tract, bringing about the development of short-chain fatty acids (SCFAs), which are known to help energy homeostasis and metabolism [20].

Various mechanisms that associate gut microbiota with the risk of obesity and metabolic disorders depend on the discoveries of rodent models, yet the structure of gut microbiota in rodents is very different from that in humans [22]. As of now, researches on gut microbiota and metabolites are still chiefly focused on obese adults, while the exploration of obese children is moderately lacking [10]. Because of the significant difference in the structure of the gut microbiota among adults and children [23], future cohort studies that concentrate on the gut microbiota and metabolites in obese children are needed. In this research, we used an in vitro fermentation approach to address the constraints of prior human examinations. An in vitro fermentation with more straightforward examinations of various dietary fiber supplements: the higher throughput of in vitro fermentation permits more extensive assortment of dietary fiber to be tried, and the impacts of these enhancements can be tried on indistinguishable microbiota tests, as opposed to after some time within subjects, which is complicated by microbiota drift over time [24], as well as contradiction in dietary composition. Using in vitro fermentation the impacts of dietary fiber on gut microbiota permits a more straightforward way to study microbial metabolite production since we can concentrate on the impacts of dietary fiber supplementation independent of the impacts of host absorption [25]. Using a preclinical in vitro fermentation model and tests from healthy children, we sought to answer three explicit lines of inquiry: (i) Can dietary fiber modify the composition, diversity, or metabolites of the gut microbiota? (ii) Will such modification affect gut metabolite production, which is probably related to childhood obesity?

2. Materials and Methods

2.1. Materials and Reagents

FOS (purity $\geq 95\%$ with $\leq 5\%$ moisture) was purchased from Quantum Hi-Tech (China) Biological Co., Ltd. (Jiangmen, Guangdong, China). Bile salt, yeast extract, L-cysteine, NaCl, KH_2PO_4 , K_2HPO_4 , heme, MgSO_4 , CaCl_2 , and corrotonic acid were purchased from Sigma Company, USA. Tryptone is derived from the trypsin hydrolysis product of bovine bone and meat and was purchased from Beijing Dingguochangsheng Biotechnology Co., Ltd.

2.2. Collection of Fresh Fecal Samples from Healthy Volunteers

The body mass index (BMI) was calculated as the ratio of the weight in kilograms (kg) to the height (h) in meters squared (m^2). The BMI for age Z scores (BFAZ) were calculated according to the criteria recommended by the WHO and then categorized as normal weight based on Chinese national standards [26]. Fresh gut samples were taken from healthy volunteers with normal weight who did not have a history of digestive diseases or any treatment with antibiotics, probiotics, or prebiotics for at least 1 month (20 boys and 20 girls, all aged between 6 and 15 years old). The volunteers had all been living in Hangzhou, Zhejiang Province, and had a decent eating routine (a balanced diet with vegetables and meat). The study was approved by the Ethical Committee of Hangzhou Centers for Disease Control and Prevention (No. 202047). Samples were collected and stored at 4°C and tested within 4 hours.

2.3. Treatment of Fresh Fecal Samples

Using three 1.5 mL sterile centrifuge tubes, we separately weighed 0.2 g of fresh fecal samples taken from fecal sampling boxes, then stored them in an 80°C freezer. 0.8 g of the fecal samples were then weighed into sterile 10 mL centrifuge tubes, 8 mL of sterile PBS buffer solution was then added, and the interfaces were fixed with tape. Lastly, a 10% fecal suspension inoculum was produced by combining the fecal and buffer solutions in the shaker and collecting the supernatant by filtering.

2.4. In Vitro Fermentation of Gut Microbiota

Gut microbiota were cultivated from fresh fecal samples utilizing a previously reported simulated gut fermentation system. [27]. The fundamental reformative YCFA medium contained the following (per 100 ml): 4.5 g/L yeast separate, 3.0 g/L tryptone, 3.0 g/L peptone, 0.4 g/L bile salt, 0.8 g/L cysteine hydrochloride, 4.5 g/L NaCl, 2.5 g/L KCl, 0.45 g/L MgCl_2 , 0.2 g/L CaCl_2 , 0.4 g/L KH_2PO_4 , 1.0 mL Tween 80, 1.0 mL resazurin, and 2.0 mL of a trace element solution. Nitrogen was then introduced to keep the fluid surface of the medium free of oxygen after dissolving and boiling. Afterwards, a peristaltic pump was used to inject 4.5 mL of YCFA medium into vials. After that, a cap was placed on the vial, and it was high-pressure steamed to sanitize it. According to Table 1, the YCFA group may add anything to YCFA medium while the FOS group must add FOS at a proportion of 8 g/100 mL.

Table 1. Characteristics of the children in different mediums

Age group	Primary school		Junior middle school	
Medium	YCFA+FOS	YCFA	YCFA+FOS	YCFA
Experimental group	FOS_P	YCFA_P	FOS_M	YCFA_M
Number of samples	20	20	20	20

2.5. Genomic DNA Extraction and 16S rRNA High-Throughput Sequencing of Gut Microbiota

The FastDNA® Spin Kit for Soil (MP Biomedicals, U.S.) was used to extract the microbial community genomic DNA from the gut microbiota in accordance with the manufacturer's

recommendations. The V3-V4 hypervariable portions of the bacterial 16S rRNA gene were amplified using the primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') using a thermocycler PCR system (GeneAmp 9700, ABI, San Diego, CA, USA). The following PCR conditions were used: 3 min of predenaturation, 27 cycles of 95 °C for 30 s each, 30 s of annealing at 55 °C, 45 s of elongation at 72 °C, and 10 min of extension at 72 °C. Majorbio Bio-Pharm Technology Co. Ltd. used a NovaSeq PE250 platform (Illumina, San Diego, USA) to pair-end sequence the purified amplicons in accordance with industry standards (Shanghai, China).

2.6. Measurement of SCFAs In Vitro Fermentation

A crotonic acid/metaphosphoric acid solution was created by dissolving 0.6464 g of crotonic acid in 100 mL of deionized water after dissolving a total of 2.5 g of metaphosphoric acid in 100 mL of deionized water. In order to complete the process of acidification in 24 h, an equal amount of the fermentation broth (500 mL) and the crotonic acid/metaphosphoric acid solution (100 mL) were combined and stored at -40 °C. Following acidification, the samples were centrifuged for three minutes at a speed of 13,000 rpm and 4 °C to separate the supernatants from the encourage, and then they were processed through a 0.22 µm hydrophilic micron membrane for separation. 150 L of the filtrate was then suctioned into a test vial.

After the sample had been placed into the gas chromatograph, the aging strategy was carried out. The heating conditions for the column were as follows: Column temperature heating conditions were as follows: column temperature: 80 °C for 1 min, 10 °C/min, increased to 190 °C and kept up with for 0.50 min; then, at that point, increased to 240 °C at a pace of 40 °C/min and kept up with for 5 min; FID detector: 240 °C; gasification chamber: 240 °C; carrier gas: nitrogen stream rate of 20 mL/min, hydrogen stream rate of 40 mL/min, air stream rate of 400 mL/min, and air stream rate of 400 mL/min. The collected data were saved.

2.7. Measurement of Gas In Vitro Fermentation

The vial was brought back to room temperature after in vitro fermentation, and the gas was then automatically analyzed by a gas analyzer to determine its composition and content.

2.8. Data Analysis

After quality control splicing, the streamlined sequences were denoised using the DADA2 plugin in the QIIME2 (version 2020.2) procedure to produce amplicon sequence variations (ASVs). Using the SILVA 16S rRNA database and the Na-ive Bayes agreement taxonomy classifier included into QIIME2, the taxonomic work for ASVs was completed (v138). The National Center for Biotechnology Information Short Read Archive has received all agreed sequencing data from raw fecal samples and fermentation samples under the accession number PRJNA938251.

The Majorbio Cloud Platform, an online resource, was used to analyze the microbiota data (www.majorbio.com). The Wilcoxon rank-sum test comparing the two groups revealed that the alpha diversity was still rising in the atmosphere. The ASV table and Bray-Curtis distance algorithm were also used as the foundation for the beta diversity (PCoA and NMDS graphs), which was used to analyze the structural changes in the microbial community at the genus level. For each category, statistical analyses of taxonomic abundances at the phylum and genus levels were conducted and presented as Venn and bar plots. Each bacterial genus in the fermentation group was evaluated using the correlation heatmap using the correlation coefficient Spearman to determine how well it correlated with SCFAs and gases. MetOrigin conducted an examination of functional prediction.

3. Results

3.1. Alpha-diversity and Beta-diversity

After in vitro fermentation, various populations of gut microbiota were examined in different populations using 16S rRNA sequencing techniques. Figure 1A–C show the alpha-diversity of the YCFA group and the FOS group, respectively. The Ace, Chao, and Shannon indices did not differ significantly ($P > 0.05$), indicating that there was little change in the community diversity between the FOS group and the YCFA group. Figure 1D,E shows the beta diversity of the FOS group and the YCFA group. During in vitro fermentation, PCoA and NMDS analyses revealed a significant difference between the FOS group and the YCFA group in the structure of the genus-level bacterial community ($P = 0.001$, Figure 1B,C).

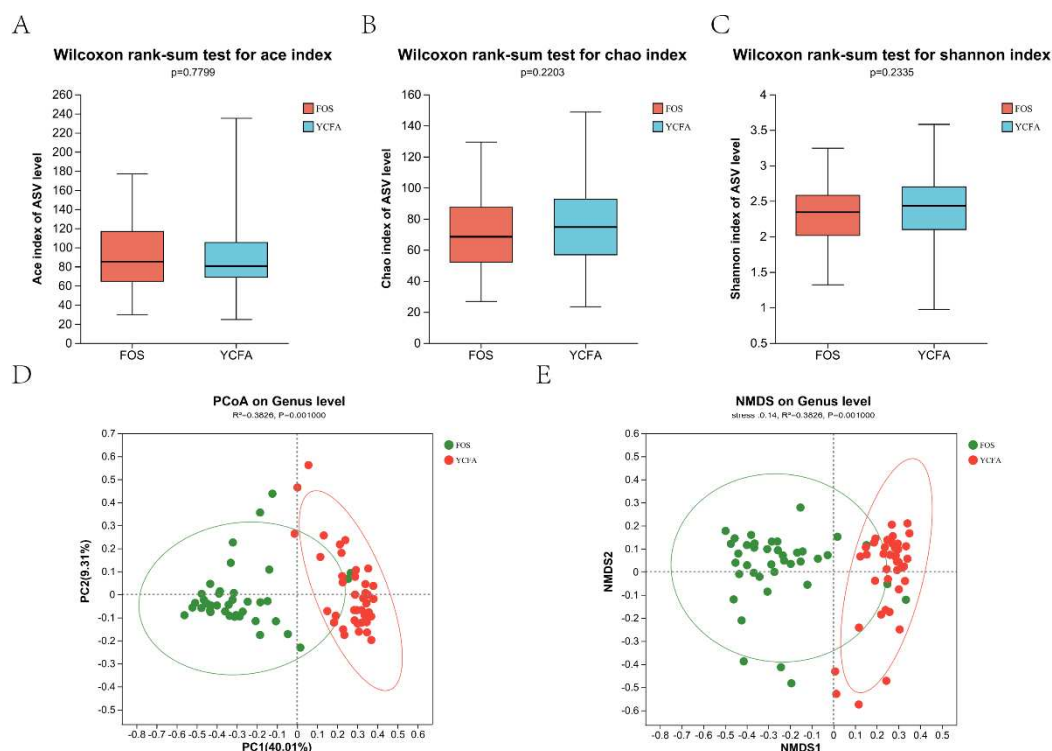


Figure 1. Diversity analysis of gut microbiota after in vitro fermentation. Alpha-diversity analysis of (A) Ace index, (B) Chao index, and (C) Shannon index between the FOS group and the YCFA group; Beta-diversity analysis of (D) PCoA and (E) NMDS between the FOS group and the YCFA group. Data are means $\pm$ SEMs (41 independent experiments $\times$ 3 replicate experiments). Statistical significance thresholds were $*0.01 < P \leq 0.05$; $**0.001 < P \leq 0.01$; $*** P \leq 0.001$.

3.2. Composition Analysis

Bifidobacterium had the highest relative abundance in the FOS group at the genus level, followed by *Escherichia-Shigella*, *Lactobacillus*, and *Bacteroides*. *Escherichia-Shigella* was the most elevated in the YCFA group, followed by *Bacteroides*, *Bifidobacterium*, and *Phascolarctobacterium* (Figure 2). After in vitro fermentation for 24 h, compared with the YCFA group, the relative abundances of *Bifidobacterium*, *Lactobacillus*, and *Faecalibacterium* were significantly expanded ($P \leq 0.05$) in the FOS group. The relative abundances of *Escherichia-Shigella*, *Bacteroides*, *unclassified_f_Enterobacteriaceae*, *Phascolarctobacterium*, and *Veillonella* were substantially lower in the FOS group than in the YCFA group (Figure 2B).

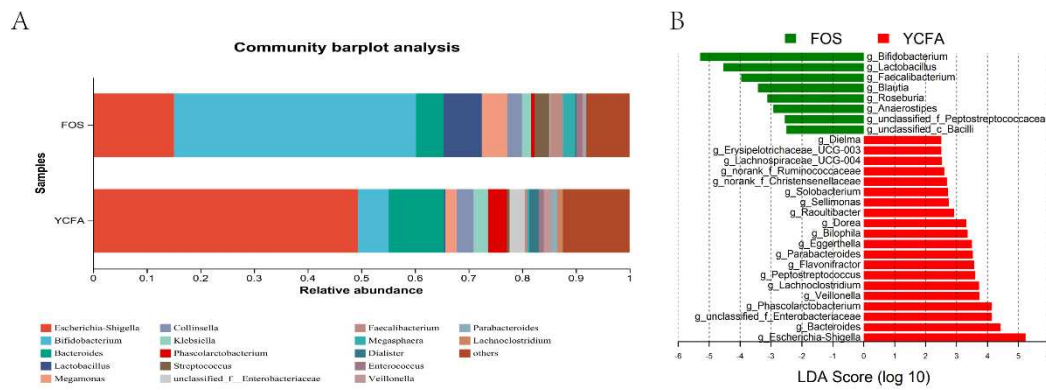


Figure 2. Composition analysis of gut microbiota after in vitro fermentation: (A) genus-level bacterial community barplot of the FOS group and the YCFA group; (B) LEfSe analysis of relative abundance at the genus level between the FOS group and the YCFA group. Data are means $\pm$ SEMs (41 independent experiments $\times$ 3 replicate experiments). Statistical significance thresholds were $*0.01 < P \leq 0.05$; $**0.001 < P \leq 0.01$; $***P \leq 0.001$.

Further analyses showed that the relative abundance of bacteria at the genus level was connected with age individually with various media (Figure 3A). We analyzed 8 bacterial genera with the most tremendous distinction in relative abundance, as shown in Figure 3B. Compared with the YCFA group, the relative abundance of *Escherichia-Shigella* ($P \leq 0.001$, Figure 3B) and *Phascolarctobacterium* ($P \leq 0.05$, Figure 3G) in the FOS group fundamentally diminished, and the relative abundance of *Bifidobacterium* ($P \leq 0.001$, Figure 3C) and *Lactobacillus* ($P \leq 0.05$, Figure 3E) essentially increased in the FOS group of the two age groups, while the relative abundance of *Bacteroides* ($P \leq 0.001$, Figure 3D) was altogether diminished exclusively in the FOS group of the primary school group, and the relative abundance of *Faecalibacterium* ($P \leq 0.001$, Figure 3H) was essentially expanded exclusively in the FOS group of the junior middle school.

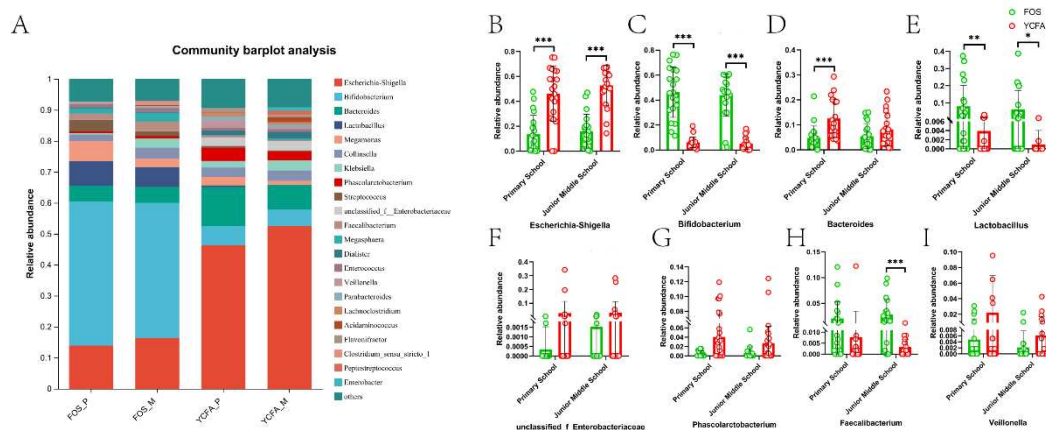


Figure 3. Bacterial composition of the primary school group and the junior middle school group on FOS medium and YCFA medium after in vitro fermentation. (A) Genus-level microbiota composition of four groups: the primary school group in the FOS+YCFA medium (FOS_P), the primary school group in the YCFA medium (YCFA_P), the junior middle school group in the FOS+YCFA medium (FOS_M), and the junior middle school group in the YCFA medium (YCFA_M); (B-I) the relative abundance of the 8 bacterial genera in the two age groups after in vitro fermentation. Data are means $\pm$ SEMs (41 independent experiments $\times$ 3 replicate experiments). Statistical significance thresholds were $*0.01 < P \leq 0.05$; $**0.001 < P \leq 0.01$; $***P \leq 0.001$.

Figure 2 consists of nine bar charts (A-I) and one heatmap (J). Panels A-I show the concentration of SCFAs and FOS/YCFA in different rumen fractions (FOS, YCFA, Ace, Pro, Isob, But, IsoV, Pen) and at different time points (Primary school, Junior middle school). Panel J is a heatmap showing the correlation between SCFAs.

Panel A: SCFAs (mM) in FOS and YCFA. FOS is significantly higher than YCFA (***).

Panel B: SCFAs (mM) in Ace, Pro, Isob, But, IsoV, and Pen. Ace is significantly higher than Pro (***), Pro is significantly higher than Isob (**), Isob is significantly higher than But (*), But is significantly higher than IsoV (*), and IsoV is significantly higher than Pen (*).

Panel C: SCFAs (mM) in FOS and YCFA. FOS is significantly higher than YCFA in Primary school (*), but not in Junior middle school (***).

Panel D: SCFAs (mM) in FOS and YCFA. FOS is significantly higher than YCFA in Primary school (***), but not in Junior middle school (***).

Panel E: SCFAs (mM) in FOS and YCFA. FOS is significantly higher than YCFA in Primary school (***), but not in Junior middle school (***).

Panel F: SCFAs (mM) in FOS and YCFA. FOS is significantly higher than YCFA in Primary school (***), but not in Junior middle school (***).

Panel G: SCFAs (mM) in FOS and YCFA. FOS is significantly higher than YCFA in Primary school (***), but not in Junior middle school (***).

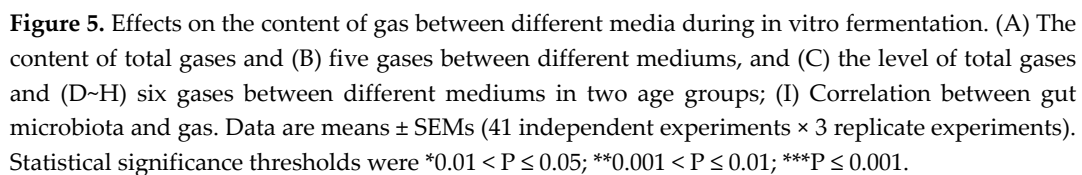
Panel H: SCFAs (mM) in FOS and YCFA. FOS is significantly higher than YCFA in Primary school (***), but not in Junior middle school (***).

Panel I: SCFAs (mM) in FOS and YCFA. FOS is significantly higher than YCFA in Primary school (***), but not in Junior middle school (***).

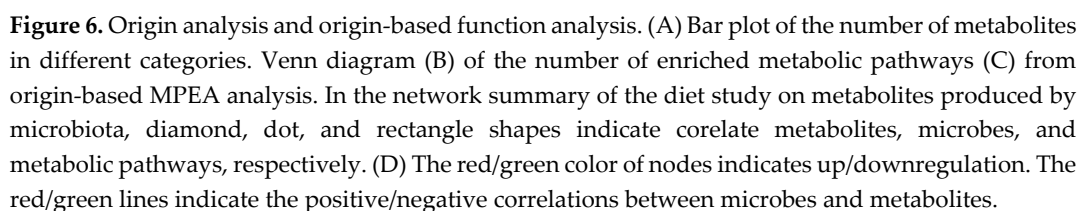
Panel J: SCFAs Correlation Heatmap. The heatmap shows the correlation between SCFAs (Ace, Pro, Isob, But, IsoV, Pen) and FOS/YCFA. The color scale ranges from -0.5 (blue) to 0.5 (red).

3.4. Gas Production During Vitro Fermentation

The composition and content of gases were analyzed after 24 h of in vitro fermentation. After 24 h of in vitro fermentation, the content of gas in the FOS group was significantly higher than that in the YCFA group ($P \leq 0.001$, Figure 5A). The FOS group and the YCFA group had the highest volume of H_2 , followed by H_2S , CH_4 , and NH_3 , and a small content of CO_2 (Figure 5B). The contents of H_2 ($P \leq 0.001$, Figure 5B), H_2S ($P \leq 0.001$, Figure 5B), and NH_3 ($P \leq 0.001$, Figure 5B) were significantly reduced in the FOS group compared to the YCFA group. Additionally, further analyses of significant differences related to age in the total gases and the single gases showed that the contents of H_2 ($P \leq 0.001$; $P \leq 0.05$, Figure 5F), H_2S ($P \leq 0.001$; $P \leq 0.001$, Figure 5G), and NH_3 ($P \leq 0.001$; $P \leq 0.05$, Figure 5H) in the FOS group of the two age groups and the content of CO_2 ($P \leq 0.01$, Figure 5H) in the FOS group of the junior middle school were significantly decreased. In analyzing the relationships between gases and gut microbiota, we employed heatmaps and Spearman correlation coefficients (Figure 5I). Among the eight bacterial genera with the highest significant difference in genus-level



Based on 16S rRNA sequencing information and metabolite information, we utilized MetOrigin to predict the metabolic function of the gut microbiota, and the results are displayed in Figure 6. A total of 9 recognized metabolites were first characterized into 3 groups: 2 bacterial metabolites, 4 bacteria-host (human) cometabolites, and 1 other (drug and food) (Figure 6A). There were 19 metabolic pathways related to bacteria-host cometabolites. (Figure 6B,C). The cometabolism network showed that 3 metabolites were related to 6 differential bacteria and 8 metabolism pathways ($P < 0.01$) (Figure 6D).



4. Discussion

This study aimed to acquire further understanding of gut microbiota dysbiosis brought about by consuming more meats low in dietary fiber in childhood and the ameliorative effect of dietary fiber on it. The communication between the gut microbiota and dietary fiber is poised to uncover the mechanistic role of the microbiota and its metabolism in childhood health and disease pathophysiology. Metabolic diseases in childhood obesity have all previously been connected to changes in the gut microbiota; however, until now, they have not been checked in an integrative manner. FOS groups (the model of dietary fiber intake) exhibit contrasts in microbiota and metabolites when contrasted with YCFA groups (the model of high meat intake) and predict the connection between the diet of children and metabolic diseases.

The importance of diet in determining the gut microbiota has long been recognized [28]. Diet has a significant effect on the composition, diversity, and richness of the gut microbiota. The gut microbiota population will change throughout time as a result of various dietary components. Long-term dietary habits, especially the consumption of animal fat and protein (*Bacteroides*) as opposed to carbohydrates or dietary fiber (*Prevotella*), are linked to what is known as childhood obesity. The *Prevotella/Bacteroides* ratio showed a dichotomy between industrialized and nonindustrialized human populations, suggesting that these bacterial populations are influenced by long-term dietary differences, such as meat (which drives *Bacteroides* in the West) and dietary fiber (which influences *Prevotella* in nonwesternized populations) [29]. In the diversity analysis in Figure 1, our results showed no significant difference in alpha diversity by comparing the FOS group with the YCFA group, while beta diversity was significantly altered, indicating that different dietary institutions can significantly affect the composition of children's gut microbiota. Our study revealed that the FOS group significantly improved the composition of children's gut microbiota compared to the YCFA group, increasing the relative abundance of probiotics and decreasing the relative abundance of harmful bacteria at the genus level. This may help lower the likelihood of childhood obesity. The significant differences between the various microbial genera were not consistent in the primary school and junior middle school groups when comparing the eight microbial genera with the greatest differences in relative abundance at various ages, comparing the FOS and YCFA groups. This was most likely caused by the small sample size and changes in the development of gut microbiota at different ages. Previous research has found dietary differences in the human gut microbiota. This high abundance of digestive *Bifidobacterium* has likewise been observed in Japanese children and is accepted to be connected to the equilibrium diet in Japan [30]. People from Western countries consume more fat and protein with less dietary fiber. Investigations have discovered that these people show a higher abundance of *Bacteroides* in their digestive microbiota. Consequently, Western diets are many times high in meat and low in dietary fiber, adjusting the metabolism of digestive microbiota and expanding the risk of obesity, cardiovascular diseases, and diabetes [31,32].

The human diet has been dependent on huge changes over the last hardly any hundred years, which is a vital consideration making sense of the enormous contrast in the predominance of chronic metabolic disease among created and emerging countries. The hole framed by decreased dietary fiber utilization has been filled by the utilization of energy-dense, high-glycemic load foods, which are a staple of the Western diet. Our progenitors are remembered to have consumed 100 g of dietary fiber daily [33]; in correlation, individuals from non-industrialized countries for the most part ingest up to 50 g daily [34], while those from industrialized western countries will more often than not consume just 12-18 g daily [35–37]. This significant difference in routine dietary fiber intake, except for the high meat content of the Western diet, is related to the distinction in gut microbiota composition and richness observed among developed and developing countries, which has been accounted for in a few studies [32,38–41]. In addition, while looking at the microbiotas of humans from various industrialized nations, there is little distinction in composition and richness [42], while vegan and vegetarian people from these social orders have a gut microbiota all the more intently looking like that of non-industrialized populaces who have high constant dietary fiber consumption [43] and low related metabolic disease predominance. Current proposals for dietary fiber intake lie somewhere in the range of 30-35 g each day for men and 25-32 g each day for women [44]. Western social orders are

as yet not complying with these rules, and low fiber utilization remains a significant general medical problem. Truth be told, even the present suggested daily intake of fiber is a lot lower than that consumed by our predecessors. Diet-microbiota collaborations are proved to be vital for the capacity of dietary fiber to work on the metabolic soundness of humans experiencing obesity and metabolic syndrome [45–48].

Dietary fiber is metabolized by a wide variety of taxa of gut microbiota to create SCFAs such as Ace, Pro, and But. The types and amounts of fiber ingested, as well as the makeup of the gut microbiota, all influence the composition and concentration of SCFAs generated by the gut microbiota. Locally and systemically, SCFAs are used as host energy substrates and signaling chemicals [49]. SCFAs also affect host metabolism by suppressing appetite, altering lipid metabolism (by boosting fat oxidation and decreasing fat synthesis and storage), and maintaining homeostasis of glucose levels [50]. Hence, SCFAs are important molecules to consider if you want to avoid or reduce obesity. In fact, scientific trials to treat obesity are now evaluating the effectiveness of SCFAs [51]. In this study, compared to the YCFA group, we found that the FOS group was able to significantly increase the content of total SCFAs, and this increase was mainly caused by Ace (Figure 4). The differential results for SCFAs were inconsistent in both age groups, except for Ace, and the production of Ace was closely related to *Bifidobacterium* and *Lactobacillus* (Figure 4). The latest research has uncovered that dietary fiber supplementation brought about upgraded microbial fermentation into Ace [52]. The role of Ace in host metabolism, including lipid synthesis, insulin secretion, hyperphagia, and obesity. Ace treatment inhibited high-fat diet-actuated body weight gain and hepatic lipid collection by upregulating articulation of hepatic Ppar α , Cpt-1, acetyl-CoA oxidase, and uncoupling protein 2 genes in mice [53].

Of the gaseous metabolites generated by protein fermentation, the concentration of NH₃ in feces might be employed as a helpful gas biomarker, as the concentration of NH₃ can rise (10–30 mmol/L) with higher than normal protein intake [54]. H₂S is another helpful indicator of protein fermentation, as its luminal concentration is elevated in those who consume a diet high in meat, and the addition of cysteine to recently passed feces in vitro significantly increases H₂S generation [54,55]. Our study showed that compared with the YCFA group, the FOS group was able to significantly decrease the concentration of total gases, and this decrease was mainly caused by H₂, NH₃, and H₂S. The differential results for gases were inconsistent in both age groups, except for NH₃ and H₂S. The consumption of NH₃ and H₂S is closely related to *Bifidobacterium*, *Lactobacillus*, and *Faecalibacterium*, and their production is highly related to *Escherichia-Shigella*, *Bacteroides unclassified_f_Enterobacteriaceae*, and *Veillonella*. Nevertheless, research into how protein fermentation affects the physiology and pathophysiology of hosts is still in its infancy, and more controlled experiments, including human intervention, are needed to gain greater knowledge.

MetOrigin is a publicly accessible and interactive online server intended for research scientists to streamline the entire course of data mining and does not need computation or bioinformatics research foundations. MetOrigin meets the pressing need to investigate the complicated connection between the microbiota and the metabolome. It is novel to perform metabolic pathway enrichment analysis on metabolites from various origins and link biological and statistically correlated microbiota. This strong bioinformatics tool provides significant data on microbial participation in metabolism and will assist researchers in finding novel dietary or pharmacological intervention systems to forestall microbial dysbiosis and keep up with metabolic equilibrium later on [56]. The outcomes of MetOrigin in this study showed that Ace had remarkable positive effects on *Bifidobacteria* but a significant negative correlation with *Bacteroides* and 4 related metabolic pathways ($P < 0.01$), including glyoxylate, dicarboxylate metabolism, and carbon fixation pathways in prokaryotes (Figure 6).

In summary, after fermentation, FOS controls the composition of the gut microbiota by hindering the growth of *Bacteroides* while increasing the abundance of *Bifidobacterium* to regulate the metabolites of the gut microbiota, including Ace, NH₃, and H₂S. Additionally, the host-specific metabolic network and microbe-metabolite affiliation network provided more unambiguous

information on metabolic changes. Dietary fiber as an intervention should be applied to high meat consumption to prevent childhood obesity.

5. Conclusions

According to our study, the fermentation of FOS altered the composition of the gut's microbial composition by raising the populations of beneficial bacteria like *Bifidobacterium* and *Faecalibacterium* while decreasing the populations of harmful bacteria like *Escherichia-Shigella* and *Bacteroides*. These increases in probiotics and decreases in harmful bacteria resulted in significant increases in short-chain fatty acid content (mainly Ace) and decreases in the production of harmful gases (mainly NH₃ and H₂S). Through MetOrigin, an interactive cloud-based platform, we predicted possible metabolic pathways by which FOS improves gut microbes and their metabolites, and the related mechanisms are likely to be applied to understand the ameliorative effects of dietary fiber on high meat consumption in children and the preventive effects on childhood obesity. These results will provide a theoretical basis for the involvement of dietary fiber in childhood precision nutrition to control childhood obesity.

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Data Availability Statement: The authors declare that the data supporting the findings of this study are presented in the manuscript. The sequences obtained in this study of 16S, and metagenomic and transcriptome data submission numbers were deposited in the NCBI Sequence Read Archive under the accession number PRJNA938251. Additional data sources are also available from the corresponding author upon request.

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