ACS OMEGA

Open Access



http://pubs.acs.org/journal/acsodf

Article

# Effects of Different Hemicellulose Components on Fermentation Kinetics and Microbial Composition in Fecal Inoculum from Suckling Piglets *In Vitro*

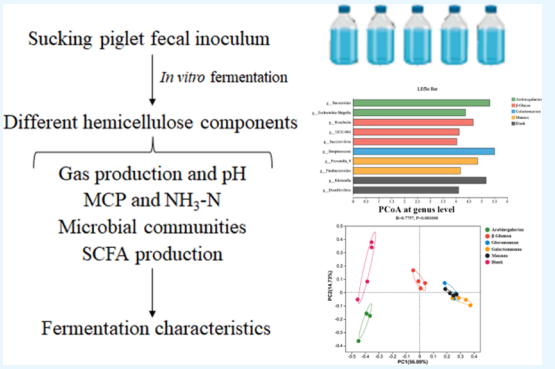
Ge Gao, Long Cai, Yuyang Fan, Roisbel Aroche Ginarte, Yanpin Li, Wenjuan Sun, Xianren Jiang, Xilong Li,* and Yu Pi*

Cite This: *ACS Omega* 2025, 10, 9120−9131

Read Online

ACCESS | Metrics & More | Article Recommendations

**ABSTRACT:** This study investigated the fermentation characteristics of different hemicellulose components using a fecal inoculum derived from suckling piglets. The results showed that after 60 h of fermentation, the arabinogalactan (Ara-gal), glucomannan (Glu-man), galactomannan (Gal-man), and mannan (Man) groups exhibited similar levels of gas production, which were higher than those of the $\beta$-glucan ($\beta$-Glu) group. The $\beta$-Glu group had the lowest pH value. After 48 h of fermentation, the Ara-gal group had the highest microbial crude protein content and the lowest ammonia nitrogen content. The Glu-man, Gal-man, and Man groups produced similar amounts of acetate, propionate, and total short-chain fatty acids (SCFAs), which were higher than those in the Ara-gal and $\beta$-Glu groups. Furthermore, the Man and Ara-gal groups showed the highest butyrate production. Significant differences in the microbial community composition were observed among the groups. Correlation analyses further revealed that the abundance of specific bacteria, such as *Prevotella_9* and *Parabacteroides*, was closely related to the production of acetate, propionate, and butyrate. These results suggest that Glu-man, Gal-man, and Man undergo rapid fermentation, with Ara-gal following, while $\beta$-Glu ferments the slowest. The distinct fiber compositions and fermentation properties of different hemicellulose components significantly influence the microbial composition and SCFA production. Our findings offer valuable theoretical insights for selecting fiber components in the diets of suckling piglets and potentially in infants.




## INTRODUCTION

Dietary fiber (DF) is defined as the indigestible portion of food derived from plants.[1] Over recent decades, an increasing amount of research has indicated that DF provides numerous health benefits to the host, including the enhancement of immune function,[2,3] modulation of gut microbial composition,[4] and promotion of weight loss.[5] The characteristics of DFs, such as hydration,[6] viscosity,[7] and fermentability,[8] determine their functions. The endogenous enzymes of monogastric animals cannot digest DF, but it is metabolized by the microbiota in the hindgut, producing short-chain fatty acids (SCFAs) that possess numerous physiological functions.[9] SCFAs, such as acetate, propionate, and butyrate, play critical roles in maintaining gut health, modulating immune responses, and serving as energy sources for colonic cells.[10] Thus, the health benefits of DF are primarily mediated through microbiota metabolism. It is now widely accepted that the composition of hindgut microorganisms and the production of SCFA are influenced by the structure and composition of DF.[11−14] Understanding these interactions is crucial for optimizing DF intake to enhance health outcomes.

The suckling period is a critical stage for mammals during which offspring rely solely on maternal milk for their nutrients.[15] Research indicates that certain oligosaccharides found in maternal milk can assist in establishing a healthy gut microbial composition in the offspring.[16] These early microbial communities are crucial for the development of the immune system and overall health. However, there is limited research exploring whether additional exogenous DF is necessary for offspring during this period. Given the rapid growth and development occurring during the newborn period, understanding the potential benefits of DF supplementation is important for improving health outcomes in suckling piglets.

Received: September 27, 2024
Revised: February 8, 2025
Accepted: February 19, 2025
Published: February 27, 2025





https://doi.org/10.1021/acsomega.4c08848
*ACS Omega* 2025, 10, 9120−9131

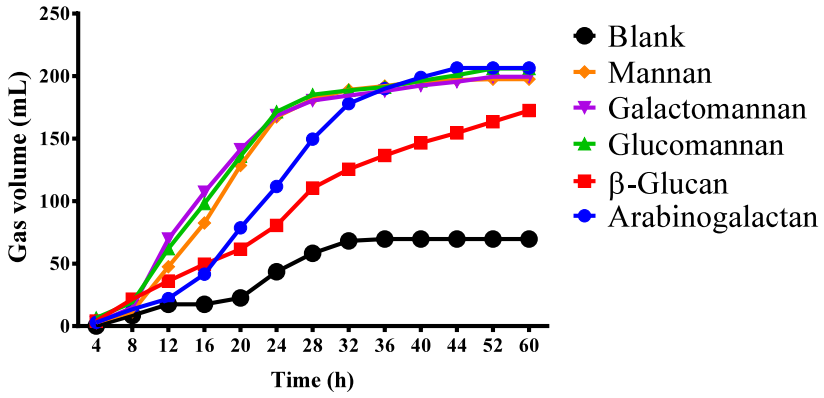



**Figure 1.** Cumulative gas production profiles of different hemicellulose components during *in vitro* fermentation by suckling piglet's fecal inoculum ($n$ = 4).

**Table 1. *In Vitro* Fermentation Cumulative Gas Production per 4 h of Different Hemicellulose Components (mL) ($n$ = 4)[a]**

| | experiment groups | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| time | Bal | Man | Gal-man | Glu-man | $\beta$-Glu | Ara-gal | SEM | $P$-value |
| 4 h | 0 | 3.0 | 2.8 | 6.5 | 4.0 | 3.0 | 0.56 | 0.08 |
| 8 h | 8.5 | 11.5 | 14.7 | 19.0 | 21.5 | 13.5 | 1.80 | 0.28 |
| 12 h | 17.5$^{e}$ | 47.5$^{bc}$ | 69.7$^{a}$ | 62.0$^{ab}$ | 36.0$^{cd}$ | 22.0$^{de}$ | 5.53 | <0.01 |
| 16 h | 17.5$^{c}$ | 82.5$^{ab}$ | 107.2$^{a}$ | 98.0$^{a}$ | 49.5$^{bc}$ | 41.5$^{c}$ | 8.85 | <0.01 |
| 20 h | 22.5$^{c}$ | 128.5$^{a}$ | 141.2$^{a}$ | 136.0$^{a}$ | 61.5$^{b}$ | 78.7$^{b}$ | 11.78 | <0.01 |
| 24 h | 43.5$^{d}$ | 167.3$^{a}$ | 168.5$^{a}$ | 171.5$^{a}$ | 80.5$^{c}$ | 111.7$^{b}$ | 13.01 | <0.01 |
| 28 h | 58.2$^{d}$ | 181.5$^{ab}$ | 180.5$^{ab}$ | 185.2$^{a}$ | 110.5$^{c}$ | 149.7$^{b}$ | 12.34 | <0.01 |
| 32 h | 68.2$^{c}$ | 189.5$^{a}$ | 184.5$^{a}$ | 188.7$^{a}$ | 125.5$^{b}$ | 178.0$^{a}$ | 11.89 | <0.01 |
| 36 h | 69.7$^{c}$ | 192.0$^{a}$ | 188.0$^{a}$ | 191.2$^{a}$ | 136.5$^{b}$ | 190.0$^{a}$ | 12.01 | <0.01 |
| 40 h | 69.7$^{c}$ | 195.8$^{a}$ | 192.5$^{a}$ | 196.2$^{a}$ | 146.5$^{b}$ | 199.0$^{a}$ | 12.38 | <0.01 |
| 44 h | 69.7$^{c}$ | 196.8$^{a}$ | 195.5$^{a}$ | 200.7$^{a}$ | 154.5$^{b}$ | 206.5$^{a}$ | 12.68 | <0.01 |
| 52 h | 69.7$^{c}$ | 197.5$^{a}$ | 199.5$^{a}$ | 206.2$^{a}$ | 163.5$^{b}$ | 206.5$^{a}$ | 12.82 | <0.01 |
| 60 h | 69.7$^{c}$ | 197.5$^{a}$ | 199.5$^{a}$ | 206.2$^{a}$ | 172.5$^{b}$ | 206.5$^{a}$ | 12.77 | <0.01 |

[a]Bal, blank; Man, mannan; Gal-man, galactomannan; Glu-man, glucomannan; $\beta$-Glu, $\beta$-glucan; Ara-gal, arabinogalactan; SEM, standard error of least squares means. The different superscript letters (a, b, c, d, e) in a row indicate significance ($P$ < 0.05). The data were analyzed using one-way ANOVA with Tukey's *post hoc* test.

DF is primarily composed of cellulose, hemicellulose, lignin, and various polysaccharides, each contributing uniquely to its health benefits. Hemicellulose is a primary component of DF and, in contrast to cellulose, comprises more diverse components and shorter linkages.[17] This structural diversity allows different types of hemicelluloses to exhibit varying physicochemical characteristics, particularly in terms of fermentability.[18] Evaluating the fermentation characteristics of DF components is an effective method for understanding their role in host health. Currently, there are two primary methodologies for studying the fermentability of DF: *in vitro* and *in vivo* approaches. *In vitro* methods, where metabolites are neither absorbed nor further metabolized, have been demonstrated to be a reliable approach for evaluating the fermentability of DF.[19] This approach allows for precise control over experimental conditions and the isolation of the effects of specific DF components on microbial activity and metabolite production.

In this study, we selected the main hemicellulose components, including arabinogalactan (Ara-gal), $\beta$-glucan ($\beta$-Glu), glucomannan (Glu-man), galactomannan (Gal-man), and mannan (Man), to investigate potential differences in their fermentation characteristics. We use indices such as gas production, SCFA production, microbial composition, microbial crude protein (MCP), and ammonia nitrogen ($NH_3$-N) to assess these characteristics. By comparing these different hemicellulose components, we aim to elucidate their distinct impacts on gut microbiota and fermentation processes, which may provide a theoretical foundation and reference for the application of DF during the breastfeeding period, ultimately contributing to better nutritional strategies and health outcomes for suckling piglets.

## RESULTS

**Differences in Cumulative Gas Production among Hemicellulose Components during Fermentation.** The cumulative gas production (GP) of different hemicellulose components during *in vitro* fermentation is shown in Figure 1 and Table 1. From 0 to 8 h, the difference in the GP was not significant among all groups. During 8 to 16 h, the Gal-man and Glu-man groups had significantly higher GP compared with $\beta$-Glu and Ara-gal groups ($P$ < 0.05). Compared with the Ara-gal group, the Man group had more GP ($P$ < 0.0.5). From 16 to 28 h, the Man, Gal-man and Glu-man groups showed similar levels of GP that were higher than those of $\beta$-Glu and Ara-gal groups ($P$ < 0.05). From 28 to 60 h, the $\beta$-Glu group had lower GP than the other hemicellulose component groups.

**Difference in pH, MCP, and $NH_3$-N among Hemicellulose Components after Fermentation.** The pH value was determined after measuring gas production, and the



https://doi.org/10.1021/acsomega.4c08848
ACS Omega 2025, 10, 9120−9131

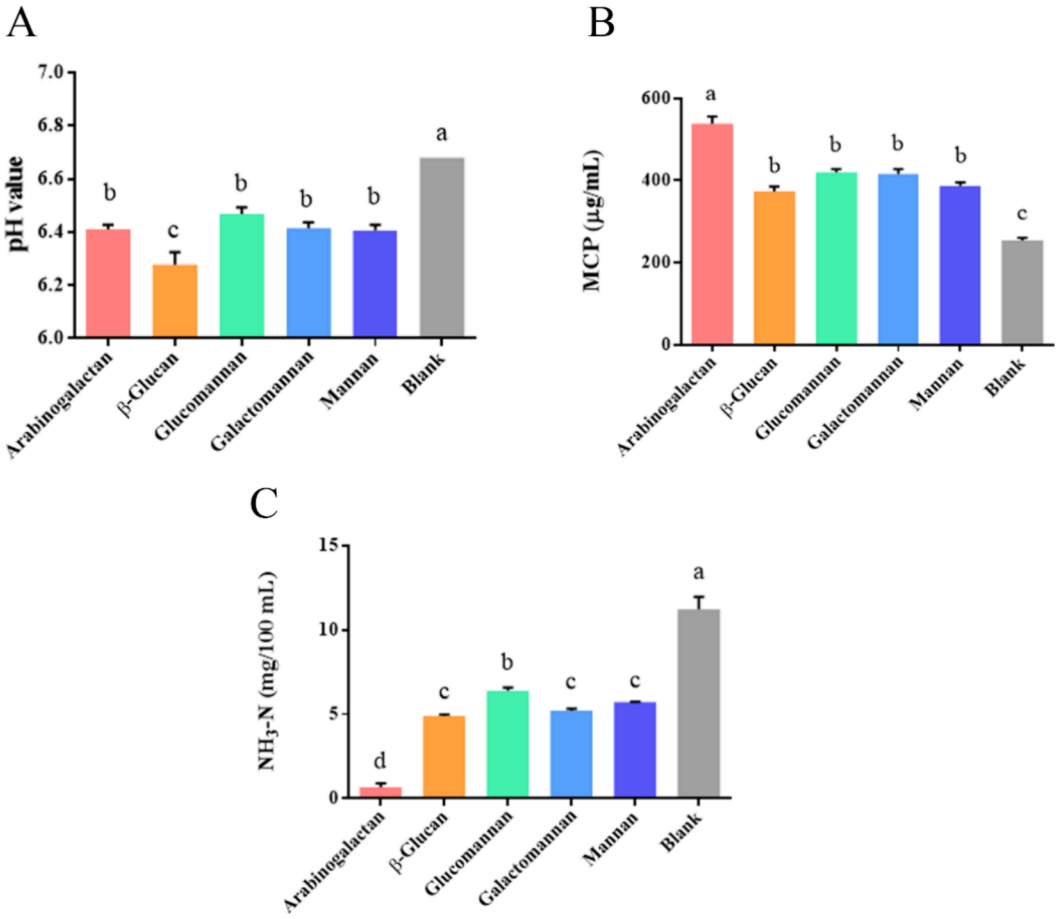



**Figure 2.** Effects of different hemicellulose components on fermentation parameters after 48 h *in vitro* fermentation by suckling piglet's fecal inoculum ($n = 4$). (A) The pH value in fermentation broth, (B) microbial crude protein (MCP) concentration, and (C) ammonia nitrogen ($NH_3$-N) concentration. The data were presented as the mean with the standard error. The different superscript letters in the graph indicate significance ($P < 0.05$).

production of MCP and $NH_3$-N was assessed after 48 h of fermentation. The results are shown in Figure 2. The $\beta$-Glu group had the lowest pH value after fermentation compared with the other hemicellulose component groups ($P < 0.05$), but the blank (Bla) group had highest pH value among all groups ($P < 0.05$) (Figure 2A). The Ara-gal group had the highest content of MCP compared to the other groups ($P < 0.05$), with the Bla group having the lowest content among all groups ($P < 0.05$) (Figure 2B). The Bla group had the highest content of $NH_3$-N among all groups ($P < 0.05$). However, among the five hemicellulose components groups, the Glu-man group had the highest content of $NH_3$-N ($P < 0.05$), while the Ara-gal group had the lowest content ($P < 0.05$) (Figure 2C).

**The Difference in SCFA Production among Hemicellulose Components after Fermentation.** The concentration of SCFA after 48 h of fermentation is demonstrated in Figure 3. The Gal-man group had a higher concentration of formate compared to the Ara-gal, Man, and $\beta$-Glu groups ($P < 0.05$). The $\beta$-Glu group had a lower ability to produce acetate compared to the other hemicellulose component groups ($P < 0.05$) (Figure 3B). The Glu-man, Gal-man, and Man groups had a higher concentration of propionate compared with the Ara-gal and $\beta$-Glu groups ($P < 0.05$) (Figure 3C). The concentration of butyrate was different among the five hemicellulose component groups, with the Ara-gal and Man groups having higher production ($P < 0.05$) and the $\beta$-Glu group having lower production ($P < 0.05$) (Figure 3D). The Glu-man, Gal-man, and Man groups had the best ability to produce SCFA after 48 h of fermentation ($P < 0.05$), while the $\beta$-Glu group had the weakest ability to produce total SCFA among the five hemicellulose components groups ($P < 0.05$) (Figure 3E). The Bla group had the lowest ability to produce acetate, propionate, butyrate, and total SCFA ($P < 0.05$).

**Difference in Microbial Diversity among Hemicellulose Components after Fermentation.** The changes in microbial diversity among different hemicellulose components in the fermentation broth are presented in Figure 4. After 48 h of fermentation, the microbial $\alpha$-diversity had significant differences among the different groups ($P < 0.05$) (Figure 4A–D). Of these, the Man, Gal-man, and Glu-man groups had higher Chao 1 and Ace indexes compared with the Ara-gal group ($P < 0.05$) (Figure 4A,B). The Ara-gal, $\beta$-Glu, and Man groups had higher Shannon index compared with the Gal-man group ($P < 0.05$) (Figure 4C). The Simpson index was highest in the Gal-man group ($P < 0.05$) and lowest in the $\beta$-Glu group ($P < 0.05$) (Figure 4D). PCoA results also indicated that the community of microbiota was distinct among different hemicellulose components at the phylum ($P = 0.001$, $R = 0.490$) and genus level ($P = 0.001$, $R = 0.776$) (Figure 4E). From the microbial community bar-lot analysis, the top eight relative abundance microbiota at the phylum level in the fermentation broth of different hemicellulose components


https://doi.org/10.1021/acsomega.4c08848
ACS Omega 2025, 10, 9120−9131

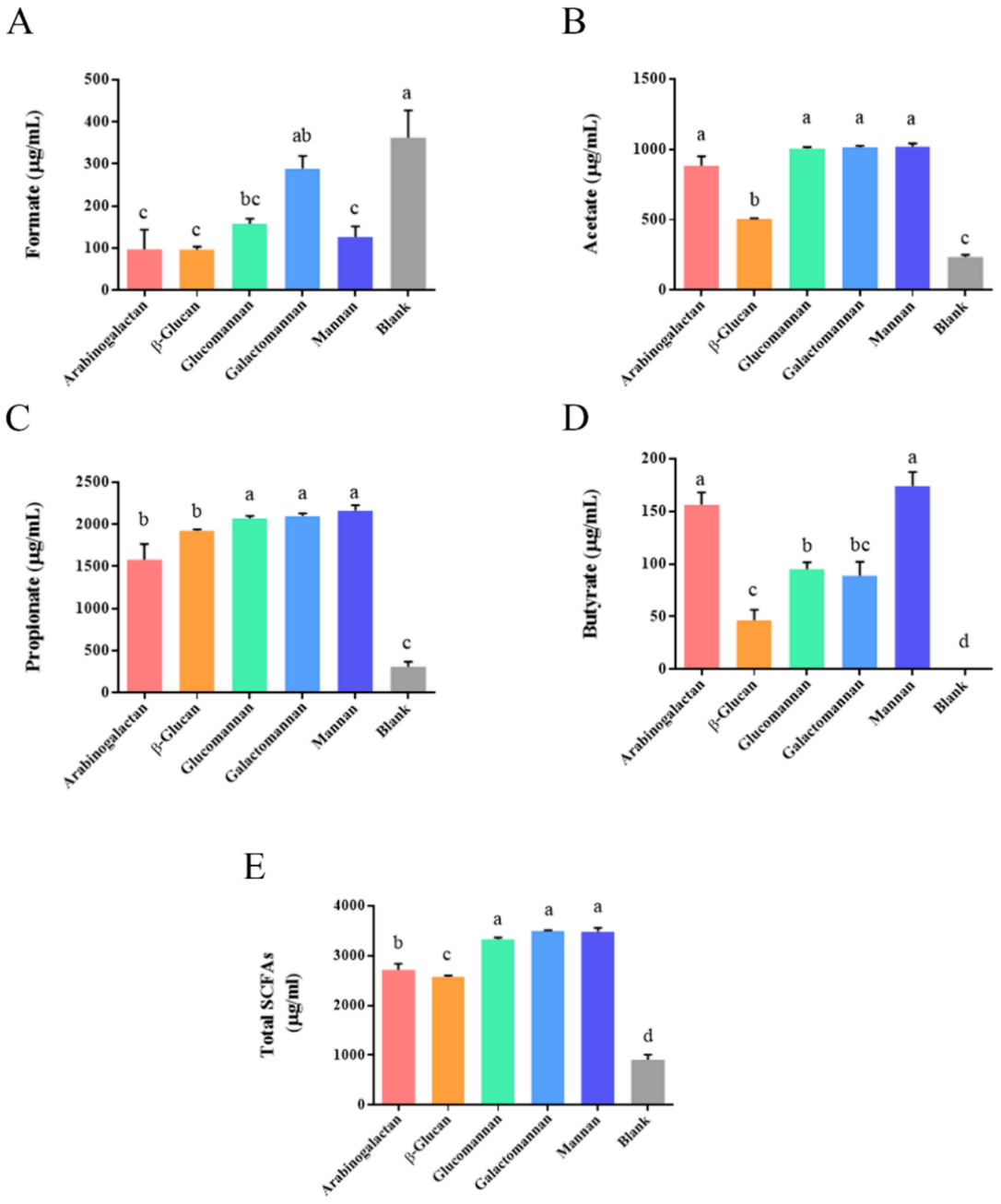



**Figure 3.** Effects of different hemicellulose components on the short-chain fatty acid (SCFA) profile after 48 h *in vitro* fermentation by suckling piglet's fecal inoculum ($n = 4$). The concentrations of (A) formate, (B) acetate, (C) propionate, (D) butyrate, and (E) total SCFA. The data were presented as the mean with standard error. The different superscript letters in the graph indicate significance ($P < 0.05$).

consist of Firmicutes, Bacteroidota, Proteobacteria, Desulfobacterota, Spirochaetota, Actinobacteriota, Fusobacteriota, and Synergistota (Figure 5A). The relative abundance of Firmicutes was higher in the Gal-man group compared with Ara-gal and Bal groups ($P < 0.05$). The relative abundance of Bacteroidota was highest in the Ara-gal group compared with the other groups ($P < 0.05$). The relative abundance of Proteobacteria was highest in the Bal group among all groups ($P < 0.05$). The relative abundance of Actinobacteriota was higher in the Glu-man group compared with $\beta$-Glu, Gal-man, and Bal groups ($P < 0.05$).

Furthermore, we analyzed the microbial community composition at the genus level, and the top 10 genera in different groups were *Streptococcus*, *Bacteroides*, *Anaerovibrio*, *Klebsiella*, *Prevotella_9*, *Phascolarctobacterium*, *Roseburia*, *Parabacteroides*, *Desulfovibrio*, and *Lachnoclostridium* (Figure 6A). The relative abundance of *Streptococcus* was higher in the Gal-man group compared with Ara-gal, $\beta$-Glu, Man, and Bal groups ($P < 0.05$) (Figure 6B). The relative abundance of *Bacteroides* and *Subdoligranulum* was higher in the Ara-gal group compared with the other groups ($P < 0.05$) (Figure 6B). The relative abundance of *Klebsiella* was highest in the Bla group compared with the other groups ($P < 0.05$) (Figure 6B). The relative abundance of *Roseburia* was highest in the $\beta$-Glu group compared with the other groups ($P < 0.05$) (Figure 6B). The relative abundance of *Parabacteroides* was higher in the Man group compared with Ara-gal and Bal groups ($P < 0.05$) (Figure 6B). The relative abundance of *Megasphaera* was


https://doi.org/10.1021/acsomega.4c08848
ACS Omega 2025, 10, 9120−9131

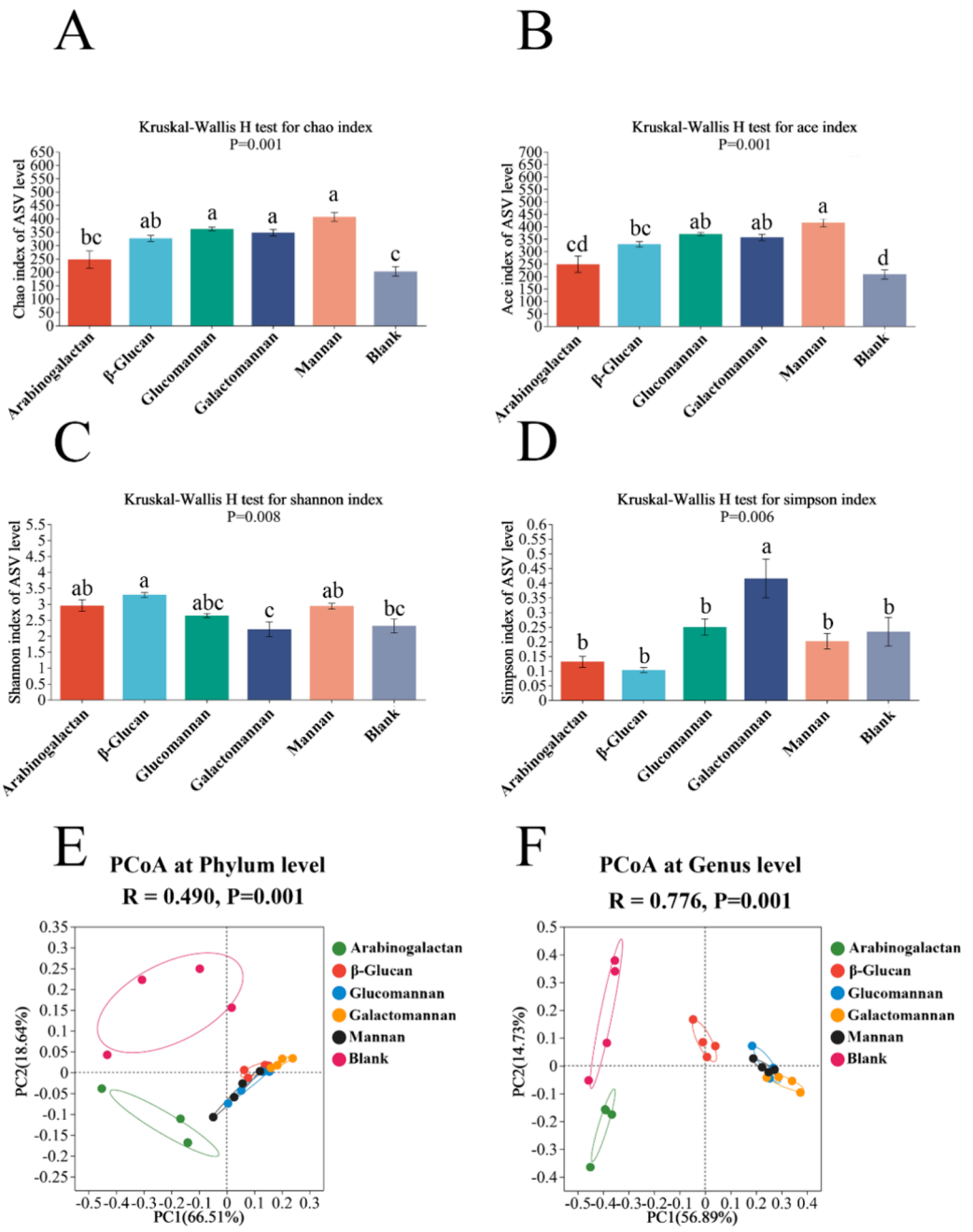



**Figure 4.** Effects of different hemicellulose components on the microbial structure after 48 h *in vitro* fermentation by suckling piglet's fecal inoculum ($n$ = 4). (A) Chao index; (B) Ace index; (C) Shannon index; (D) Simpson; and PCoA based on the (E) phylum and (F) genus levels. The result was analyzed by the Kruskal−Wallis test and PCoA plot based on the Bray−Curtis distance matrix. The data were presented as the mean with standard error. The different superscript letters in the graph indicate significance ($P$ < 0.05).

higher in Gal-man compared with Ara-gal and Bal groups ($P$ < 0.05) (Figure 6B). The relative abundance of *Succinivibrio* was higher in $\beta$-Glu compared with Ara-gal, Glu-man, Man, and Bal groups ($P$ < 0.05) (Figure 6B). LefSe analysis indicated that there were two, three, one, two, and two biomarkers, respectively, in the Ara-gal, $\beta$-Glu, Gal-man, Man, and Bla groups, with *Bacteroides*, *Roseburia*, *Succinivibrio*, *Streptococcus*, *Prevotella_9*, and *Klebsiella* as the dominant genera (Figure 6C).

**The Correlation between SCFA, MCP, $NH_3$-N, and Microbial Communities.** The correlation between the concentrations of SCFA, MCP, and $NH_3$-N and the top 10 relative abundance genera was obtained via Spearman's correlation analysis (Figure 7). The result indicated that the content of formate was positively associated with the relative abundance of *Desulfovibrio* and negatively associated with *Bacteroides*. The contents of acetate and propionate were positively associated with the relative abundance of *Streptococcus*, *Prevotella_9*, and *Parabacteroides* and negatively associated with *Bacteroides*, *Anaerovibrio*, and *Klebsiella*. The content of butyrate was positively associated with the relative abundance of *Prevotella_9* and *Parabacteroide* and negatively associated with *Klebsiella* and *Desulfovibrio*. MCP was strongly negative with a relative abundance of *Desulfovibrio*. $NH_3$-N was strongly positively associated with *Desulfovibrio*.



https://doi.org/10.1021/acsomega.4c08848
ACS Omega 2025, 10, 9120−9131

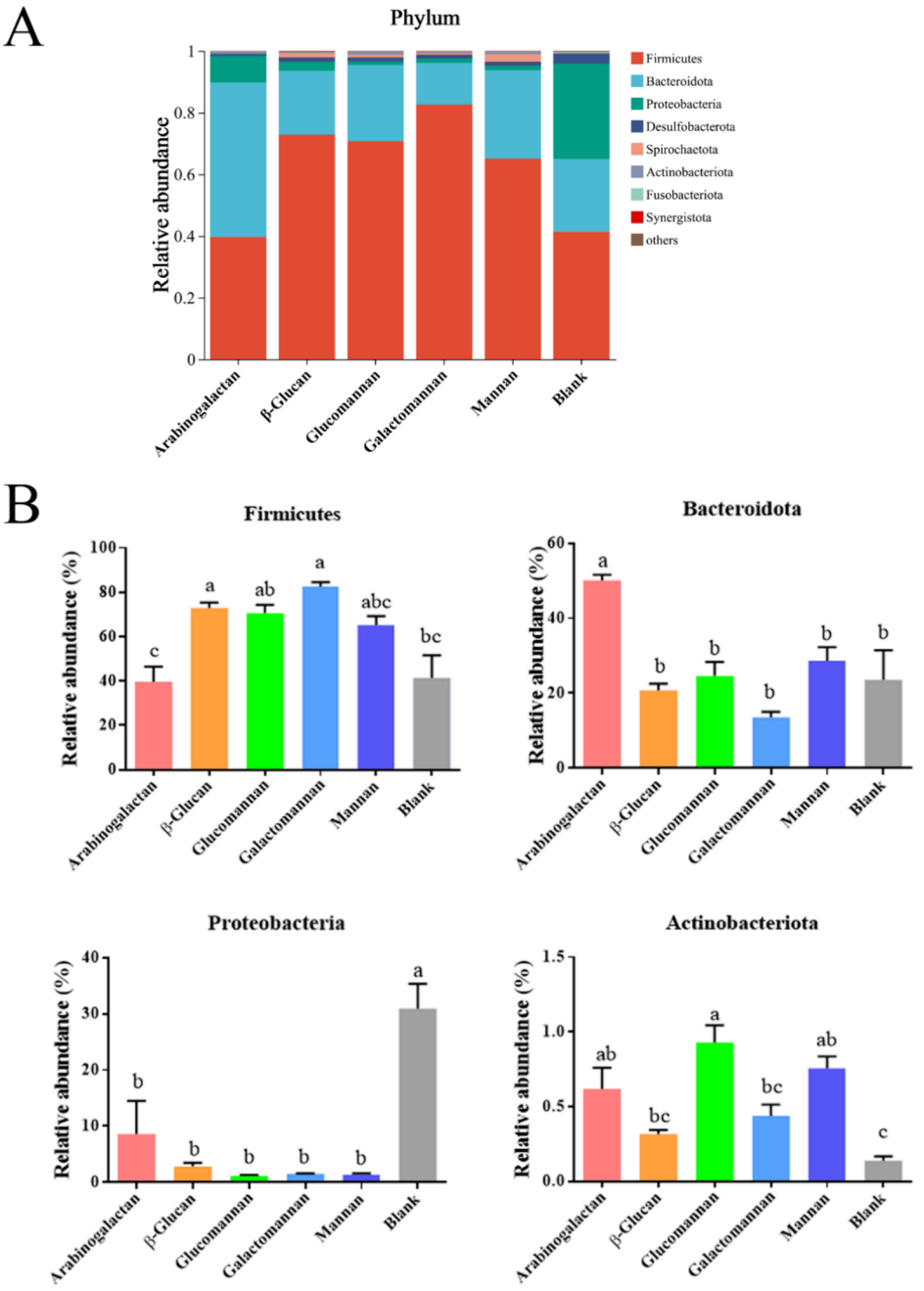



**Figure 5.** Effects of different hemicellulose components on microbial composition at the phylum level after 48 h *in vitro* fermentation by suckling piglet's fecal inoculum ($n$ = 4). (A) Relative abundance of microbiota among different components at the phylum level. (B) The main abundant genera in the gut microbiota are at the phylum level in the fermentation broth. The different superscript letters in the graph indicate significance ($P$ < 0.05).

## DISCUSSION

Hemicellulose is a significant component of DF, representing the second most abundant type within the DF spectrum. Certain components of hemicellulose, such as xylooligosaccharides and mannooligosaccharides, have been reported to function as prebiotics.[20] Consequently, hemicellulose components possess significant potential as prebiotics. The suckling period is a crucial stage for mammals, as offspring depend entirely on maternal milk for nutrition. Studies have shown that specific oligosaccharides in milk play a key role in promoting a healthy gut microbiota in the young.[15,16] Some reports suggest that supplementing suckling piglets with galactooligosaccharides or fructooligosaccharides can increase the abundances of *Lactobacillus* and *Bifidobacterium*, indicating that hemicellulose can enhance gut microbiota.[21,22] However, which specific type of hemicellulose is most effective in fostering a healthier gut environment in suckling piglets. Therefore, a deeper understanding of their fermentation characteristics during the suckling period may provide valuable insights into their potential application in improving health outcomes in suckling piglets.

**Gas Production.** The volume of gas produced during fermentation serves as an indicator of the extent of *in vitro* fermentation, with a shorter time to reach the gas production plateau indicating faster fermentation. The composition of



https://doi.org/10.1021/acsomega.4c08848
ACS Omega 2025, 10, 9120−9131

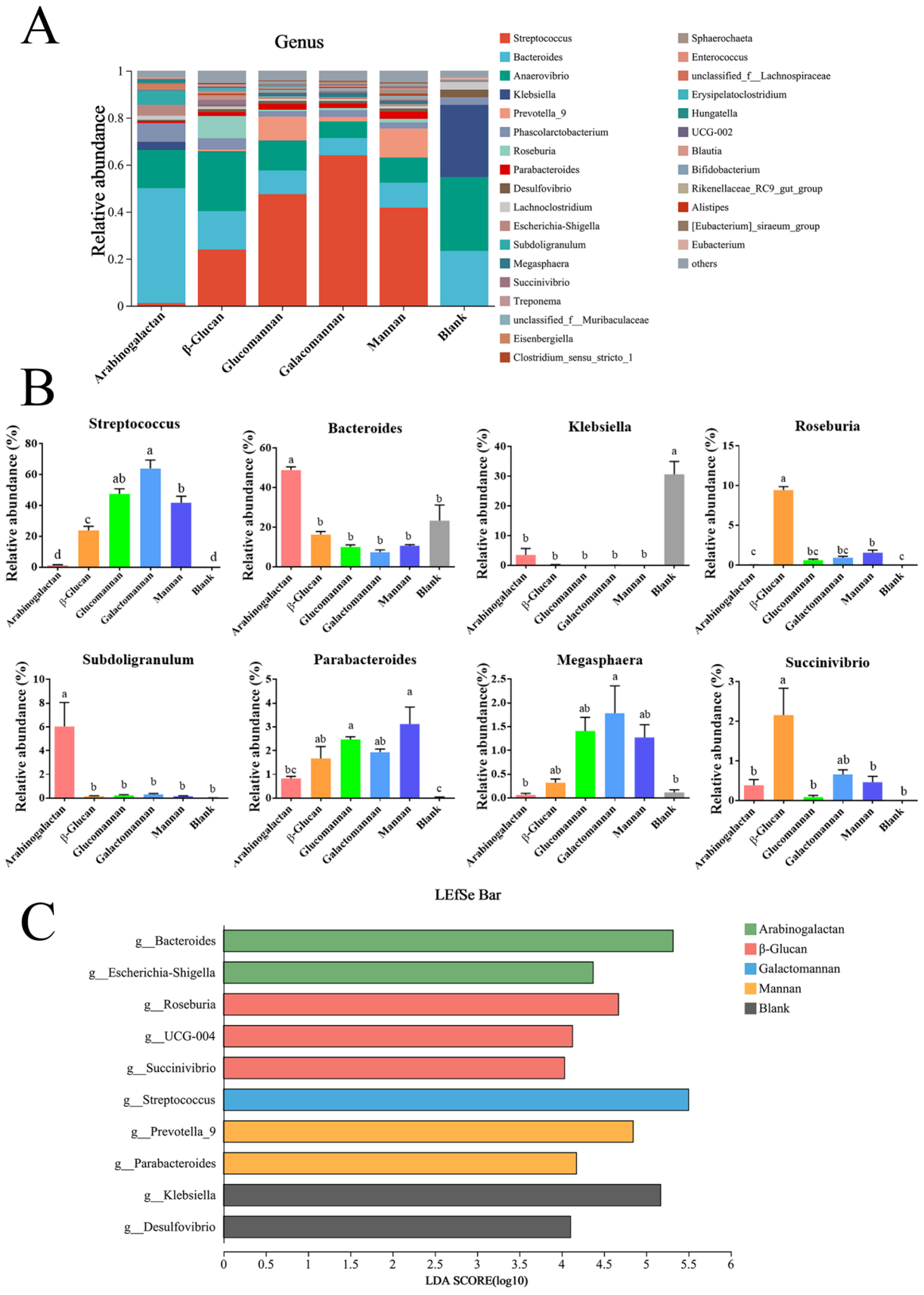



**Figure 6.** Relative abundance of main bacteria in different hemicellulose components' fermentation broth after 48 h fermentation at the genus level ($n$ = 4). (A) Relative abundance of microbiota among different components at the genus level. (B) The bacteria in the top eight significantly changed at the genus level. (C) Histograms of a linear discriminant analysis (LDA) score (threshold > 4.0). The different superscript letters in the graph indicate significance ($P < 0.05$).



https://doi.org/10.1021/acsomega.4c08848
ACS Omega 2025, 10, 9120−9131

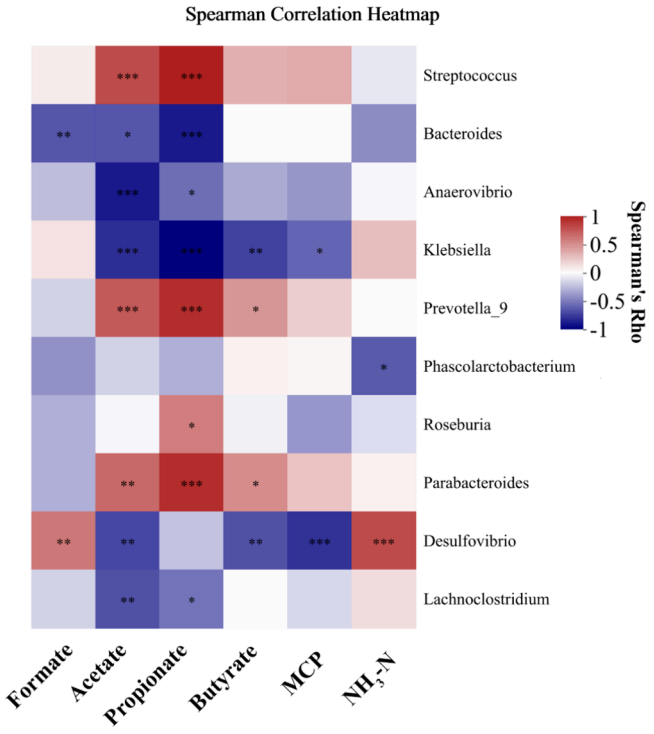



**Figure 7.** Spearman's correlation between the concentrations of SCFA, MCP, and $NH_3$-N and the relative abundances of the top 10 bacteria at the genus level in different hemicellulose components after 48 h fermentation. SCFA, short chain fatty acid; MCP, microbial crude protein; $NH_3$-N, ammonia nitrogen. *$P < 0.05$; **$P < 0.01$; and ***$P < 0.001$.

gases mainly includes hydrogen, carbon dioxide, and methane.[23,24] In the present study, we found that the total gas production after fermentation is similar among the Man, Gal-Man, Glu-Man, and Ara-gal groups. However, during fermentation, the rate of gas production differed among the groups. The $\beta$-Glu group demonstrated the lowest gas production capacity, with fermentation remaining incomplete even after 60 h. Galactomannan, glucomannan, mannan, and arabinogalactan were classified as soluble hemicelluloses, whereas $\beta$-glucan was classified as insoluble. It is reported that gas production is mainly influenced by fermentation substrates and microbial populations,[18,25] and soluble fiber typically undergoes faster fermentation than insoluble fiber.[26] This explains why $\beta$-glucan exhibited the poorest ability to produce gas within a 60 h fermentation period.

**MCP and $NH_3$-N Changes.** MCP and $NH_3$-N are two classical indexes to indicate how microbiota utilize nitrogen sources. In our study, we supplied the same amount of $NH_4HCO_3$ as the nitrogen sources, which is a form of $NH_3$-N. The result of our research demonstrated that the Ara-gal group can utilize most of $NH_3$-N and convert it into MCP, and the other hemicellulose components showed similar results but were not as strong as arabinogalactan. This is not surprising, as when hemicellulose components are supplied, microbiota accelerate the conversion of $NH_3$-N to MCP because hemicellulose components provide sufficient carbon sources to meet the microbiota growth requirements. In the present study, there was a special phenomenon: compared with other hemicellulose components, the Ara-gal group significantly increased the conversion of $NH_3$-N to MCP. Considering that arabinogalactan is composed of arabinose and galactose and mother's milk contains a large amount of lactose, we hypothesize that after long-term breastfeeding, the microbes in the intestines of piglets adapt to the daily intake of lactose, making galactose utilization more efficient. However, this hypothesis still needs further investigation to be proven. In another aspect, the conversion of $NH_3$-N to MCP reflected the speed of microbiota growth, indicating that arabinogalactan has the potential to speed up the early establishment of gut microbiota in suckling piglets.

**pH Changes.** The pH of the fermentation broth is primarily altered by metabolites such as SCFA and lactic acid produced by microbiota, serving as another key indicator reflecting fermentation progress.[27] In our research, the $\beta$-Glu group had the lowest pH value after 60 h of fermentation, indicating that $\beta$-glucan can produce more acid metabolites. It has been reported that while soluble fiber ferments faster than insoluble fiber, insoluble fibers have a greater ability to produce SCFA than soluble fibers.[28,29] This research could explain why, after 60 h of fermentation, the $\beta$-Glu group had the lowest pH value.

**SCFA Production.** SCFA is the end metabolite of the microbe utilizing DF, which is considered the main "bridge" connecting the microbe to host health. It has been proven that SCFA has numerous functions for host health, including anti-inflammatory,[30] antitumor,[31] and immunomodulatory effects.[32] SCFAs include formate, acetate, propionate, butyrate, and valeric acid, each serving different roles in host health. The production and composition of SCFA are mainly determined by various DF and microbiota community compositions. Different microbiota have different abilities to produce different types of SCFA. For example, *Clostridium butyricum* can produce butyrate, whereas most *Lactobacillus* species cannot. Thus, the species of SCFA are mainly influenced by the microbiota composition. It is reported that with increasing fermentation time, the content of SCFA also increases until the fermentation is completed.[20] Thus, the production of SCFA is mainly related to the degree of fiber utilization. In the present study, after 48 h of fermentation, we observed that different hemicellulose components had differences in the SCFA production or composition. The $\beta$-Glu group had the poorest SCFA production, especially in acetate and butyrate, compared with the other groups. As mentioned above, this kind of $\beta$-glucan is insoluble, and the fermentation speed is slower than that in other groups; therefore, the SCFA concentration is lower than that in other groups.

In our study, there is an interesting phenomenon: those hemicellulose components composed of mannose have the same production levels of acetate, propionate, and total SCFA and are higher than those of other groups. This result may indicate that mannose may be easily utilized and produce more SCFAs in suckling piglets. Among SCFAs, butyrate is the most attractive component to study. Butyrate not only has important immunomodulatory functions but can also be absorbed by the colonic epithelium as an energy source.[33] In our study, the Ara-gal and Man groups had the greatest production of butyrate among all of the groups. Considering the beneficial function of butyrate, arabinogalactan and mannan hold potential as prebiotics that can be added to formula milk for suckling piglets.

**Microbial Community Dynamics and Diversity.** The composition of pig intestinal microbiota can be modified by DF with different structures.[34] Generally, the easy fermentation of fiber within a certain time could provide more carbon


https://doi.org/10.1021/acsomega.4c08848
ACS Omega 2025, 10, 9120−9131

sources to microbes,[35] which may increase the microbial diversity and alter the microbial composition. In the present study, the hemicellulose components composed of mannose exhibited $\alpha$-diversity higher than that of other ingredients. Those groups have faster fermentation ability, as mentioned above, so they can quickly degrade into monosaccharides, which are utilized by the microbes, leading to higher diversity. It is reported that glucomannan, galactomannan, linear mannan, and galactoglucomannan are the four subfamilies of the mannan family.[36] Thus, these mannan polysaccharides have great similarities in their backbone. This may explain why these three hemicellulose components have similarities in fermentation characteristics and microbial composition.

In the present study, the result of PCoA indicated that the community has significant separation among hemicellulose components, demonstrating that different components shaped the different compositions. This finding is consistent with the previous study.[18] It is reported that the microbial community of pigs is mainly composed of Firmicutes, Bacteroidota, and Proteobacteria.[37] Our results concurred with this observation. Our research further revealed that the different hemicellulose components altered the microbial composition at both the phylum and genus level. Specifically, the Ara-gal group was found to increase the abundance of Bacteroidota. It is well-known that microorganisms have competition and cross-feeding among different species.[38] In our study, this situation of competition is much more obvious. At the genus level, we found that the abundance of *Streptococcus* was significantly higher in the Gal-man, Glu-man, and Man groups and even more than 60% in the Gal-man group. Meanwhile, the abundance of *Bacteroides* was >50% in the Ara-gal group. These findings suggested that *Streptococcus* could rapidly utilize galactomannan, glucomannan, and mannan, giving them a growth advantage. Similarly, *Bacteroides* demonstrated the same trend in arabinogalactan. *Streptococcus* is often considered to be pathogenic. To determine whether the enriched *Streptococcus* in our study was harmful, we used the ASV sequence and compared it with the NCBI database. The result indicated that the enriched species was *Streptococcus alactolyticus* (identification:100%). There is limited research on this bacterium in pigs, but some articles have evaluated the safety and antioxidant activity of *Streptococcus alactolyticus*, demonstrating its safety and potential as a probiotic.[39,40] Using the same method, we found that *Bacteroides* in the Ara-gal group were mainly composed of *Bacteroides thetaiotaomicron* (identification:100%), which is recognized as an efficient polysaccharides degrader.[41] *Roseburia* is considered to be the main butyrate producer.[42] Numerous studies have proven that *Roseburia* could produce butyrate to maintain host health.[43,44] In our study, we observed the highest abundance of *Roseburia* in the $\beta$-Glu group, which aligns with another study,[45,46] indicating that *Roseburia* has a preference for insoluble $\beta$-glucan. The potential mechanism is that the insoluble $\beta$-glucan is difficult to hydrolyze, and only bacteria with the ability to degrade and utilize these substrates can grow rapidly. The presence of flagella in *Roseburia* is proposed to enhance its chemotaxis and attachment to insoluble substrates, which may explain why *Roseburia* can be enriched in the substrate.[47,48] *Klebsiella* and *Desulfovibrio* are conditional pathogenic bacteria. Our study found that the Bla group, devoid of hemicellulose components, had a higher abundance of these bacteria. This observation implies that pathogenic bacteria can thrive even in nutrient-sparse settings, underscoring the necessity to furnish ample nutrients to support the growth of beneficial bacteria, thereby ensuring that they can outcompete harmful pathogens. This observation further highlights the critical role of certain hemicellulose components in fostering a conducive gut microbiome for suckling piglets, potentially improving their health and growth performance.

## CONCLUSIONS

Distinct differences in gas production; pH; MCP, $NH_3$-N, and SCFA concentrations; and microbial compositions were observed during the fermentation of arabinogalactan, $\beta$-glucan, glucomannan, galactomannan, and mannan using a fecal inoculum from suckling piglets. Glucomannan, galactomannan, and mannan were rapidly fermented by the microbiota, evident from their high production of gas and SCFA. Arabinogalactan exhibited moderate fermentability, while $\beta$-glucan was the least fermentable. Notably, $\beta$-glucan fermentation promoted the growth of *Roseburia*. These findings suggested that different hemicellulose components exert diverse physiological functions in regulating the host nutrition and health. This study provides new insights into the potential of different hemicellulose types with varying physicochemical properties, to regulate the microbial composition and SCFA production, ultimately supporting or improving gut health in suckling piglets.

## MATERIALS AND METHODS

**Substrates.** In this study, we used commercial-grade hemicellulose components. Arabinogalactan (originating from corn, arabinose/galactose = 1:2.6 to 1:3.6, molecular weight: 38 kDa), $\beta$-glucan (originating from yeast, molecular weight: 500 kDa), glucomannan (originating from oats, glucose/mannose = 1:1.6 to 1:2, molecular weight: 2000 kDa), galactomannan (originating from corn, galactose/mannose = 1:2 to 1:4, molecular weight: 200 kDa), and mannan (originating from *Irpex lacteus*, molecular weight: 90 kDa) were purchased from Shaanxi Pioneer Biotech Co., Ltd. (Xi'an, China). The purity of these hemicellulose components was 90, 80, 98, 90, and 98%, respectively.

**Preparation of Fecal Inoculum.** The process of inoculum preparation was adapted from previous studies with modifications.[26,49] Compared with growing pigs, suckling piglets have minimal excretion. Therefore, 50 healthy suckling piglets (Duroc × Landrace × Large white, age 7−10 days, without consuming creep feed) were selected to provide the feces for the fecal inoculum. Feces were collected directly from the anus of each piglet and stored in plastic bags prefilled with $CO_2$. All samples of feces were diluted with a prewarmed buffer solution (1:5, w/v) consisting of 1× PBS and 15% glycerine (v/v). The diluted mixture was stirred for 5 min using a homogenizer and filtered through four layers of sterile gauze. The filtered liquid served as the inoculum. All processes were conducted in an anaerobic chamber filled with $CO_2$. The inoculum was dispensed into 50 mL centrifuge tubes and then stored at −80 °C until further fermentation experiment.

**Cumulative Gas Production Trial.** Different hemicellulose components (arabinogalactan, $\beta$-glucan, glucomannan, galactomannan, and mannan) were accurately weighed at 0.5 g and used as substrates for fermentation. The medium for gas production and *in vitro* fermentation was prepared based on a previous study.[35] Briefly, the medium was formulated with 8.32 g/L $NaHCO_3$, 0.95 g/L $NH_4HCO_3$, 1.36


https://doi.org/10.1021/acsomega.4c08848
ACS Omega 2025, 10, 9120−9131

g/L $Na_2HPO_4$, 1.47 g/L $KH_2PO_4$, 0.14 g/L $MgSO_4·7H_2O$, 0.30 g/L $Na_2S·9H_2O$, 76.09 mg/L NaOH, 15.69 mg/L $CaCl_2·2H_2O$, 11.89 mg/L $MnCl_2·4H_2O$, 1.19 mg/L $CoCl_2·6H_2O$, 9.51 mg/L $FeCl_3·6H_2O$, and 1.19 mg/L resazurin. The substrates were placed flat in 250 mL fermentation bottles and blended with 82 mL of the medium. After filling the medium, the bottles were flushed with $CO_2$ for 10 s and sealed. All of the bottles were inoculated with 5 mL of inoculum using a 5 mL injector and then incubated at 39 °C for 60 h in a temperature-controlled incubator. Each hemicellulose component was analyzed in four replicates. After fermentation, a pH meter (FE28-Standard, Mettler-Toledo, Switzerland) was used to detect the pH value of the fermentation broth. The blank control was the bottles without a substrate. All of the processes were performed under the same conditions. The volume of gas production (GP) was measured every 4 h using a method based on a previous study.[49]

***In Vitro* Fermentation Trial.** Another trial was designed for *in vitro* fermentation, and the conditions of *in vitro* fermentation were identical to those employed in the gas production trial. Samples were collected at 48 h based on the result of gas production ($n$ = 4). After incubation, the fermentation bottles were placed in ice water to stop the fermentation process for 30 min. All bottles were shaken and divided into three parts in 10 mL sterile tubes for further analysis. Then, the remaining liquid was used to determine the pH value.

**Microbial Crude Protein and Ammonia Nitrogen Content.** The microbial crude protein (MCP) content of the fermentation liquid was determined using the colorimetric method.[50] The ammonia nitrogen ($NH_3$-N) content was analyzed using the indophenol method.[51]

**SCFA Concentration.** The SCFA concentration in the fermentation liquid was detected using ion chromatography following the methodology outlined in a previous study.[52] Briefly, 2 mL of fermentation broth was centrifuged at 10,000$g$ for 10 min. The resulting supernatants were then filtered through a 0.22 $\mu$m membrane and diluted with distilled water in a ratio of 1:4. The prepared samples were subsequently analyzed for SCFA using an ion chromatograph (IC; Metrohm, Switzerland).

**Bacterial Community.** The total microbial genomic DNA in the fermentation broth was extracted using a commercial kit (Omega Bio-Tek, Norcross, GA, USA) following the instructions of the manufacturer. The hypervariable regions V3−V4 of the bacterial 16S rRNA gene were amplified using specific primer pairs 341F (5′-ACTCCTACGGGAGGCAG-CAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The PCR products were purified with a commercial kit (Axegen Biosciences, Union City, CA, USA). The purified amplicons were then sequenced on a specific platform (Illumina, San Diego, CA, USA). Under the default parameters, the results were denoised by DADA2, and then an amplicon sequence variant (ASV) was generated.[53] All ASVs were classified using the Silva 138 database via a Naive Bayes classifier implemented in the QIIME 2 software. Alpha diversity was calculated with Mothur (ver. 1.30.2), while beta diversity was assessed using the vegan package (version 3.3.1). Principal coordinate analysis (PCoA) was employed using Bray−Curtis distances to elucidate compositional dissimilarities among microbial communities, complemented by the analysis of similarities (ANOSIM) test to assess statistically significant differences. The linear discriminant analysis effect size (LEfSe) with a threshold greater than 4.0 was employed to study the differences in microbiota composition among the hemicellulose components. Data analysis was conducted online in the Majorbio I-Sanger Cloud Platform (https://www.i-sanger.com/). The raw data were uploaded to the NCBI Sequence Read Archive (SRA) database, and the accession number is PRJNA1152294.

**Statistical Analysis.** The differences in gas production and MCP, $NH_3$-N, and SCFA concentration among different hemicellulose components were analyzed using one-way ANOVA with Tukey's *post hoc* test using SPSS 19.0 (IBM, Armonk, NY, USA). The differences in key altered genera among hemicellulose components were determined using the Kruskal−Wallis test followed by the Scheffe *post hoc* test. Spearman's correlation coefficients were used to analyze the relationship between gas production and MCP, $NH_3$-N, SCFA, and microbial communities. Data are presented as the mean ± the standard error. A $P < 0.05$ was considered statistically significant.

## ■ ASSOCIATED CONTENT

### Data Availability Statement

The data supporting this study's findings are openly available in NCBI at PRJNA1152294.

## ■ AUTHOR INFORMATION


### Corresponding Authors

**Xilong Li** − *Key Laboratory of Feed Biotechnology of Ministry of Agriculture and Rural Affairs, Institute of Feed Research, Chinese Academy of Agricultural Sciences, Beijing 100081, China*; Phone: +86-010-82108134; Email: lixilong@caas.cn

**Yu Pi** − *Key Laboratory of Feed Biotechnology of Ministry of Agriculture and Rural Affairs, Institute of Feed Research, Chinese Academy of Agricultural Sciences, Beijing 100081, China*; orcid.org/0000-0003-1320-0290; Email: piyu@caas.cn

### Authors

**Ge Gao** − *Key Laboratory of Feed Biotechnology of Ministry of Agriculture and Rural Affairs, Institute of Feed Research, Chinese Academy of Agricultural Sciences, Beijing 100081, China*

**Long Cai** − *Key Laboratory of Feed Biotechnology of Ministry of Agriculture and Rural Affairs, Institute of Feed Research, Chinese Academy of Agricultural Sciences, Beijing 100081, China*

**Yuyang Fan** − *Key Laboratory of Feed Biotechnology of Ministry of Agriculture and Rural Affairs, Institute of Feed Research, Chinese Academy of Agricultural Sciences, Beijing 100081, China*

**Roisbel Aroche Ginarte** − *Key Laboratory of Feed Biotechnology of Ministry of Agriculture and Rural Affairs, Institute of Feed Research, Chinese Academy of Agricultural Sciences, Beijing 100081, China; Department of Animal Husbandry, Faculty of Agricultural Science, University of Granma, Bayamo 85100, Cuba*

**Yanpin Li** − *Key Laboratory of Feed Biotechnology of Ministry of Agriculture and Rural Affairs, Institute of Feed Research, Chinese Academy of Agricultural Sciences, Beijing 100081, China*

**Wenjuan Sun** − *Key Laboratory of Feed Biotechnology of Ministry of Agriculture and Rural Affairs, Institute of Feed*




https://doi.org/10.1021/acsomega.4c08848
ACS Omega 2025, 10, 9120−9131

*Research, Chinese Academy of Agricultural Sciences, Beijing 100081, China*

**Xianren Jiang** − *Key Laboratory of Feed Biotechnology of Ministry of Agriculture and Rural Affairs, Institute of Feed Research, Chinese Academy of Agricultural Sciences, Beijing 100081, China;* orcid.org/0000-0002-8799-3572

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.4c08848

### Author Contributions

G.G. and L.C. participated in the experimental design, performed the experiment, and analyzed the data. G.G. and Y.F. were responsible for data analysis and drafted the manuscript. G.G., R.G., Y.L., W.S., Y.P., and X.J. assisted in the experiment procedures. X.L. and Y.P. supervised the concept, contributed to the supply design, reviewed and edited the manuscript, and participated in the entire research process. All authors approved the final version of the manuscript submitted for publication.

### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

This study was financially supported by the China Postdoctoral Science Foundation (2023M730594), the Agricultural Science and Technology Innovation Program of the Feed Research Institute of the Chinese Academy of Agricultural Sciences (CAAS-IFR-ZDRW202402), the National Key Research and Development Program of China (2021YFD1300202), and the Central Public-interest Scientific Institution Basal Research Fund (1610382023011).

## ■ ABBREVIATIONS

Ara-gal, arabinogalactan; $\beta$-Glu, $\beta$-glucan; Glu-man, glucomannan; Gal-man, galactomannan; Man, mannan; MCP, microbial protein; $NH_3$-N, ammonia nitrogen; SCFA, short-chain fatty acid; DF, dietary fiber; GP, gas production

## ■ REFERENCES

(1) Jarrett, S.; Ashworth, C. J. The role of dietary fibre in pig production, with a particular emphasis on reproduction. *J. Anim. Sci. Biotechnol.* **2018**, *9* (1), 59.

(2) Tan, J. K.; Macia, L.; Mackay, C. R. Dietary fiber and SCFAs in the regulation of mucosal immunity. *Journal of Allergy and Clinical Immunology* **2023**, *151* (2), 361−370.

(3) So, D.; Whelan, K.; Rossi, M.; Morrison, M.; Holtmann, G.; Kelly, J. T.; Shanahan, E. R.; Staudacher, H. M.; Campbell, K. L. Dietary fiber intervention on gut microbiota composition in healthy adults: a systematic review and meta-analysis. *American Journal of Clinical Nutrition* **2018**, *107* (6), 965−983.

(4) Solah, V.; Kerr, D.; Hunt, W. J.; Johnson, S.; Boushey, C.; Delp, E.; Meng, X.; Gahler, R.; James, A.; Mukhtar, A.; Fenton, H.; Wood, S. Effect of Fibre Supplementation on Body Weight and Composition, Frequency of Eating and Dietary Choice in Overweight Individuals. *Nutrients* **2017**, *9* (2), 149.

(5) Venter, C.; Meyer, R. W.; Greenhawt, M.; Pali-Schöll, I.; Nwaru, B.; Roduit, C.; Untersmayr, E.; Adel-Patient, K.; Agache, I.; Agostoni, C.; Akdis, C. A.; Feeney, M.; Hoffmann-Sommergruber, K.; Lunjani, N.; Grimshaw, K.; Reese, I.; Smith, P. K.; Sokolowska, M.; Vassilopoulou, E.; Vlieg-Boerstra, B.; Amara, S.; Walter, J.; O'Mahony, L. Role of dietary fiber in promoting immune health—An EAACI position paper. *Allergy* **2022**, *77* (11), 3185−3198.

(6) Luo, X.; Wang, Q.; Zheng, B.; Lin, L.; Chen, B.; Zheng, Y.; Xiao, J. Hydration properties and binding capacities of dietary fibers from bamboo shoot shell and its hypolipidemic effects in mice. *Food Chem. Toxicol.* **2017**, *109*, 1003−1009.

(7) Chen, M.; Guo, L.; Nsor-Atindana, J.; Goff, H. D.; Zhang, W.; Zhong, F. The effect of viscous soluble dietary fiber on nutrient digestion and metabolic responses II: In vivo digestion process. *Food Hydrocolloid* **2020**, *107*, No. 105908.

(8) Bai, Y.; Zhao, J. b.; Tao, S. y.; Zhou, X. j.; Pi, Y.; Gerrits, W. J. J.; Johnston, L. J.; Zhang, S. y.; Yang, H. j.; Liu, L.; Zhang, S.; Wang, J. j. Effect of dietary fiber fermentation on short-chain fatty acid production and microbial composition in vitro. *J. Sci. Food Agr* **2020**, *100* (11), 4282−4291.

(9) Xu, T.; Wu, X.; Liu, J.; Sun, J.; Wang, X.; Fan, G.; Meng, X.; Zhang, J.; Zhang, Y. The regulatory roles of dietary fibers on host health via gut microbiota-derived short chain fatty acids. *Current Opinion in Pharmacology* **2022**, *62*, 36−42.

(10) Sivaprakasam, S.; Bhutia, Y. D.; Yang, S. P.; Ganapathy, V. Short-Chain Fatty Acid Transporters: Role in Colonic Homeostasis. *Compr Physiol* **2017**, *8* (1), 299−314.

(11) Ye, S.; Shah, B. R.; Li, J.; Liang, H.; Zhan, F.; Geng, F.; Li, B. A critical review on interplay between dietary fibers and gut microbiota. *Trends in Food Science & Technology* **2022**, *124*, 237−249.

(12) Tanabe, K.; Nakamura, S.; Moriyama-Hashiguchi, M.; Kitajima, M.; Ejima, H.; Imori, C.; Oku, T. Dietary Fructooligosaccharide and Glucomannan Alter Gut Microbiota and Improve Bone Metabolism in Senescence-Accelerated Mouse. *J. Agr Food Chem.* **2019**, *67* (3), 867−874.

(13) Lange, K.; Hugenholtz, F.; Jonathan, M. C.; Schols, H. A.; Kleerebezem, M.; Smidt, H.; Müller, M.; Hooiveld, G. J. E. J. Comparison of the effects of five dietary fibers on mucosal transcriptional profiles, and luminal microbiota composition and SCFA concentrations in murine colon. *Molecular Nutrition & Food Research* **2015**, *59* (8), 1590−1602.

(14) Bai, Y.; Zhou, X.; Zhao, J.; Wang, Z.; Ye, H.; Pi, Y.; Che, D.; Han, D.; Zhang, S.; Wang, J. Sources of Dietary Fiber Affect the SCFA Production and Absorption in the Hindgut of Growing Pigs. *Front. Nutr.* **2022**, *8*, No. 719935.

(15) Garwolińska, D.; Namieśnik, J.; Kot-Wasik, A.; Hewelt-Belka, W. Chemistry of Human Breast Milk—A Comprehensive Review of the Composition and Role of Milk Metabolites in Child Development. *J. Agr Food Chem.* **2018**, *66* (45), 11881−11896.

(16) Selma-Royo, M.; González, S.; Gueimonde, M.; Chang, M.; Fürst, A.; Martínez-Costa, C.; Bode, L.; Collado, M. C. Maternal Diet Is Associated with Human Milk Oligosaccharide Profile. *Mol. Nutr. Food Res.* **2022**, *66* (15), 2200058.

(17) Gao, Y.; Guo, M.; Wang, D.; Zhao, D.; Wang, M. Advances in extraction, purification, structural characteristics and biological activities of hemicelluloses: A review. *Int. J. Biol. Macromol.* **2023**, *225*, 467−483.

(18) Bai, Y.; Zhou, X.; Li, N.; Zhao, J.; Ye, H.; Zhang, S.; Yang, H.; Pi, Y.; Tao, S.; Han, D.; Zhang, S.; Wang, J. In Vitro Fermentation Characteristics and Fiber-Degrading Enzyme Kinetics of Cellulose, Arabinoxylan, $\beta$-Glucan and Glucomannan by Pig Fecal Microbiota. *Microorganisms* **2021**, *9* (5), 1071.

(19) Wang, M.; Wichienchot, S.; He, X.; Fu, X.; Huang, Q.; Zhang, B. In vitro colonic fermentation of dietary fibers: Fermentation rate, short-chain fatty acid production and changes in microbiota. *Trends in Food Science & Technology* **2019**, *88*, 1−9.

(20) Pi, Y.; Hu, J.; Bai, Y.; Wang, Z.; Wu, Y.; Ye, H.; Zhang, S.; Tao, S.; Xiao, Y.; Han, D.; Ni, D.; Zou, X.; Wang, J. Effects of dietary fibers with different physicochemical properties on fermentation kinetics and microbial composition by fecal inoculum from lactating sows in vitro. *J. Sci. Food Agr* **2021**, *101* (3), 907−917.

(21) Zhou, J.; Wang, Y.; Zeng, X.; Zhang, T.; Li, P.; Yao, B.; Wang, L.; Qiao, S.; Zeng, X. Effect of antibiotic-free, low-protein diets with specific amino acid compositions on growth and intestinal flora in weaned pigs. *Food Funct* **2020**, *11* (1), 493−507.

(22) Wang, J.; Tian, S. Y.; Yu, H.; Wang, J.; Zhu, W. Y. Response of Colonic Mucosa-Associated Microbiota Composition, Mucosal Immune Homeostasis, and Barrier Function to Early Life

Galactooligosaccharides Intervention in Suckling Piglets. *J. Agr Food Chem.* **2019**, *67* (2), 578−588.
(23) Judd, L. M.; Kohn, R. A. Test of conditions that affect in vitro production of volatile fatty acids and gases1. *J. Anim Sci.* **2018**, *96* (2), 694−704.
(24) Wannapokin, A.; Ramaraj, R.; Whangchai, K.; Unpaprom, Y. Potential improvement of biogas production from fallen teak leaves with co-digestion of microalgae. *3 Biotech* **2018**, *8* (2), 123.
(25) Fagundes, G. M.; Benetel, G.; Santos, K. C.; Welter, K. C.; Melo, F. A.; Muir, J. P.; Bueno, I. C. S. Tannin-Rich Plants as Natural Manipulators of Rumen Fermentation in the Livestock Industry. *Molecules* **2020**, *25* (12), 2943.
(26) Tao, S. Y.; Bai, Y.; Zhou, X. J.; Zhao, J. B.; Yang, H. J.; Zhang, S.; Wang, J. J. In Vitro Fermentation Characteristics for Different Ratios of Soluble to Insoluble Dietary Fiber by Fresh Fecal Microbiota from Growing Pigs. *Acs Omega* **2019**, *4* (12), 15158−15167.
(27) Hua, M.; Lu, J.; Qu, D.; Liu, C.; Zhang, L.; Li, S.; Chen, J.; Sun, Y. Structure, physicochemical properties and adsorption function of insoluble dietary fiber from ginseng residue: A potential functional ingredient. *Food Chem.* **2019**, *286*, 522−529.
(28) Zhao, J.; Bai, Y.; Tao, S.; Zhang, G.; Wang, J.; Liu, L.; Zhang, S. Fiber-rich foods affected gut bacterial community and short-chain fatty acids production in pig model. *Journal of Functional Foods* **2019**, *57*, 266−274.
(29) Zhao, J.; Bai, Y.; Zhang, G.; Liu, L.; Lai, C. Relationship between Dietary Fiber Fermentation and Volatile Fatty Acids' Concentration in Growing Pigs. *Animals* **2020**, *10* (2), 263.
(30) Perego, S.; Sansoni, V.; Banfi, G.; Lombardi, G. Sodium butyrate has anti-proliferative, pro-differentiating, and immunomodulatory effects in osteosarcoma cells and counteracts the TNFα-induced low-grade inflammation. *Int. J. Immunopathol. Pharmacol.* **2018**, *31*, No. 0394632017752240.
(31) Kuefer, R.; Hofer, M. D.; Altug, V.; Zorn, C.; Genze, F.; Kunzi-Rapp, K.; Hautmann, R. E.; Gschwend, J. E. Sodium butyrate and tributyrin induce in vivo growth inhibition and apoptosis in human prostate cancer. *Br. J. Cancer* **2004**, *90* (2), 535−541.
(32) Feingold, K. R.; Moser, A.; Shigenaga, J. K.; Grunfeld, C. Inflammation stimulates niacin receptor (GPR109A/HCA2) expression in adipose tissue and macrophages. *J. Lipid Res.* **2014**, *55* (12), 2501−2508.
(33) Zhu, L.-B.; Zhang, Y.-C.; Huang, H.-H.; Lin, J. Prospects for clinical applications of butyrate-producing bacteria. *World Journal of Clinical Pediatrics* **2021**, *10* (5), 84−92.
(34) Jonathan, M. C.; van den Borne, J. J. G. C.; van Wiechen, P.; Souza da Silva, C.; Schols, H. A.; Gruppen, H. In vitro fermentation of 12 dietary fibres by faecal inoculum from pigs and humans. *Food Chem.* **2012**, *133* (3), 889−897.
(35) Jana, U. K.; Kango, N.; Pletschke, B. Hemicellulose-Derived Oligosaccharides: Emerging Prebiotics in Disease Alleviation. *Front. Nutr.* **2021**, *8*, No. 670817.
(36) de O. Petkowicz, C. L.; Reicher, F.; Chanzy, H.; Taravel, F. R.; Vuong, R. Linear mannan in the endosperm of. *Carbohyd. Polym.* **2001**, *44* (2), 107−112.
(37) Kim, H. B.; Isaacson, R. E. The pig gut microbial diversity: Understanding the pig gut microbial ecology through the next generation high throughput sequencing. *Vet. Microbiol.* **2015**, *177* (3−4), 242−251.
(38) Coyte, K. Z.; Rakoff-Nahoum, S. Understanding Competition and Cooperation within the Mammalian Gut Microbiome. *Curr. Biol.* **2019**, *29* (11), R538−R544.
(39) Zhang, J.; Zhang, H.; Wang, L.; Zhang, K.; Qiu, Z.; Zhang, K.; Yue, C.; Zhang, Y.; Zhao, X.; Li, J. The safety and potential probiotic properties analysis of Streptococcus alactolyticus strain FGM isolated from the chicken cecum. *Ann. Microbiol.* **2021**, *71* (1), 19.
(40) Gu, X.; Wang, H.; Wang, L.; Zhang, K.; Tian, Y.; Wang, X.; Xu, G.; Guo, Z.; Ahmad, S.; Egide, H.; Liu, J.; Li, J.; Savelkoul, H. F. J.; Zhang, J.; Wang, X. The antioxidant activity and metabolomic analysis of the supernatant of Streptococcus alactolyticus strain FGM. *Sci. Rep.* **2024**, *14* (1), 8413.
(41) Ye, M.; Yu, J.; Shi, X.; Zhu, J.; Gao, X.; Liu, W. Polysaccharides catabolism by the human gut bacterium -Bacteroides thetaiotaomicron: advances and perspectives. *Critical Reviews in Food Science and Nutrition* **2021**, *61* (21), 3569−3588.
(42) Louis, P.; Flint, H. J. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiology Letters* **2009**, *294* (1), 1−8.
(43) Zhao, C.; Bao, L.; Qiu, M.; Wu, K.; Zhao, Y.; Feng, L.; Xiang, K.; Zhang, N.; Hu, X.; Fu, Y. Commensal cow Roseburia reduces gut-dysbiosis-induced mastitis through inhibiting bacterial translocation by producing butyrate in mice. *Cell Rep.* **2022**, *41* (8), No. 111681.
(44) Machiels, K.; Joossens, M.; Sabino, J.; De Preter, V.; Arijs, I.; Eeckhaut, V.; Ballet, V.; Claes, K.; Van Immerseel, F.; Verbeke, K.; Ferrante, M.; Verhaegen, J.; Rutgeerts, P.; Vermeire, S. A decrease of the butyrate-producing speciesRoseburia hominisandFaecalibacterium prausnitziidefines dysbiosis in patients with ulcerative colitis. *Gut* **2014**, *63* (8), 1275−1283.
(45) Fehlbaum, S.; Prudence, K.; Kieboom, J.; Heerikhuisen, M.; Van den Broek, T.; Schuren, F. H. J.; Steinert, R. E.; Raederstorff, D. In Vitro Fermentation of Selected Prebiotics and Their Effects on the Composition and Activity of the Adult Gut Microbiota. *Int. J. Mol. Sci.* **2018**, *19* (10), 3097.
(46) Gao, Y.; Yu, L.; Ye, Z.; Zhang, C.; Gong, Y.; Zhang, Q.; Zhang, C.; Zhao, J.; Narbad, A.; Chen, W.; Zhai, Q.; Tian, F. In vitro batch fermentation demonstrates variations in the regulation of gut microbiota and metabolic functions by beta-glucans of differing structures. *Food Res. Int.* **2024**, *186*, No. 114287.
(47) Scott, K. P.; Martin, J. C.; Chassard, C.; Clerget, M.; Potrykus, J.; Campbell, G.; Mayer, C. D.; Young, P.; Rucklidge, G.; Ramsay, A. G.; Flint, H. J. Substrate-driven gene expression in Roseburia inulinivorans: importance of inducible enzymes in the utilization of inulin and starch. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108 Suppl 1* (Suppl 1), 4672−4679.
(48) Van den Abbeele, P.; Belzer, C.; Goossens, M.; Kleerebezem, M.; De Vos, W. M.; Thas, O.; De Weirdt, R.; Kerckhof, F. M.; Van de Wiele, T. Butyrate-producing Clostridium cluster XIVa species specifically colonize mucins in an in vitro gut model. *ISME J.* **2013**, *7* (5), 949−61.
(49) Luo, C.; Duan, J.; Zhong, R.; Liu, L.; Gao, Q.; Liu, X.; Chen, L.; Zhang, H. In vitro fermentation characteristics of different types of fiber-rich ingredients by pig fecal inoculum. *J. Sci. Food Agr* **2024**, *104* (9), 5296−5304.
(50) Cui, J. H.; Yang, H. J.; Yu, C. Q.; Bai, S.; Wu, T. T.; Song, S. S.; Sun, W.; Shao, X. M.; Jiang, L. S. Effect of urea fertilization on biomass yield, chemical composition, rumen digestibility and fermentation characteristics of straw of highland barley planted in Tibet. *J. Agr Sci-Cambridge* **2016**, *154* (1), 151−164.
(51) Le, P. T. T.; Boyd, C. E. Comparison of Phenate and Salicylate Methods for Determination of Total Ammonia Nitrogen in Freshwater and Saline Water. *J. World Aquacult Soc.* **2012**, *43* (6), 885−889.
(52) Cai, L.; Gao, G.; Yin, C.; Bai, R.; Li, Y.; Sun, W.; Pi, Y.; Jiang, X.; Li, X. The Effects of Dietary Silybin Supplementation on the Growth Performance and Regulation of Intestinal Oxidative Injury and Microflora Dysbiosis in Weaned Piglets. *Antioxidants* **2023**, *12* (11), 1975.
(53) Callahan, B. J.; McMurdie, P. J.; Rosen, M. J.; Han, A. W.; Johnson, A. J. A.; Holmes, S. P. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **2016**, *13* (7), 581−583.


https://doi.org/10.1021/acsomega.4c08848
*ACS Omega* 2025, 10, 9120−9131