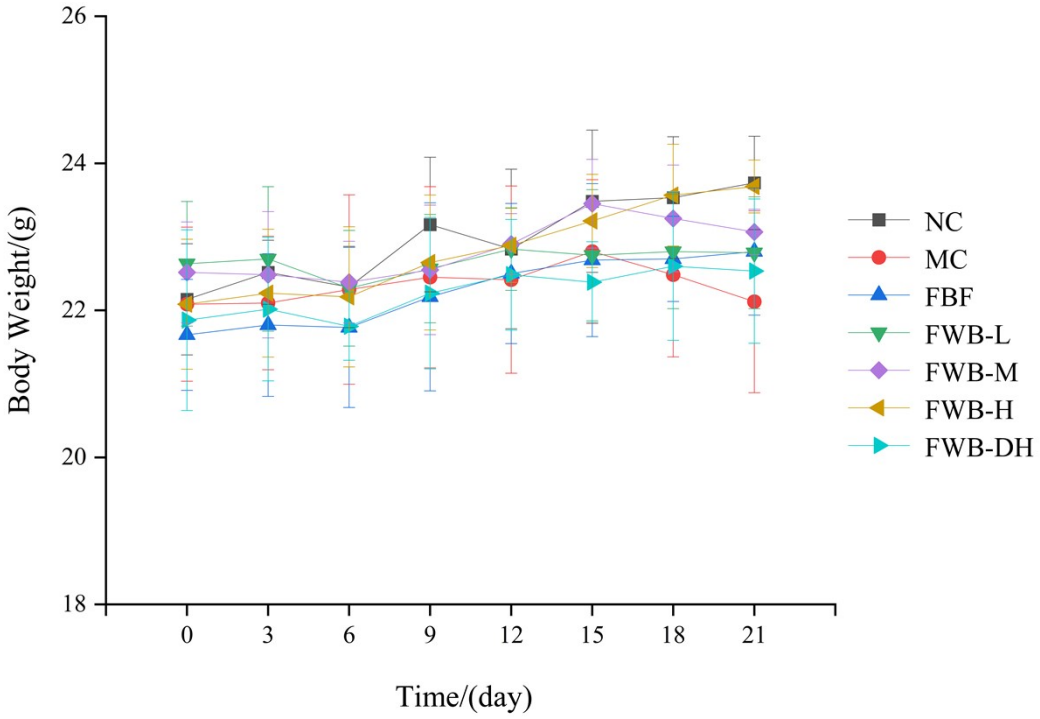
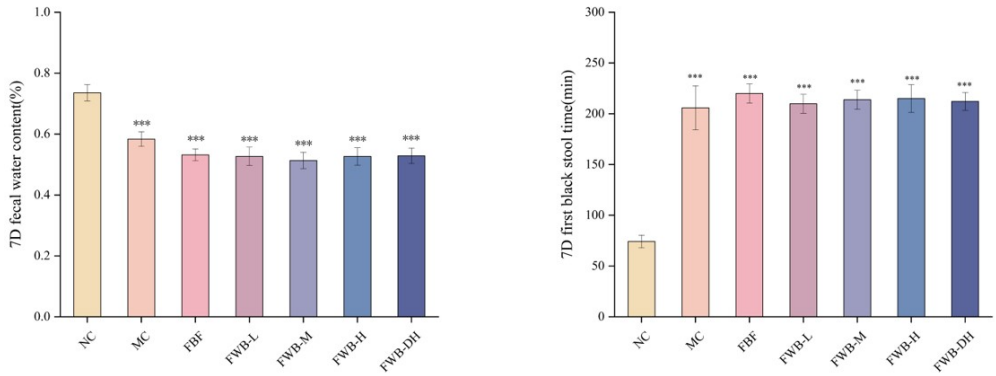
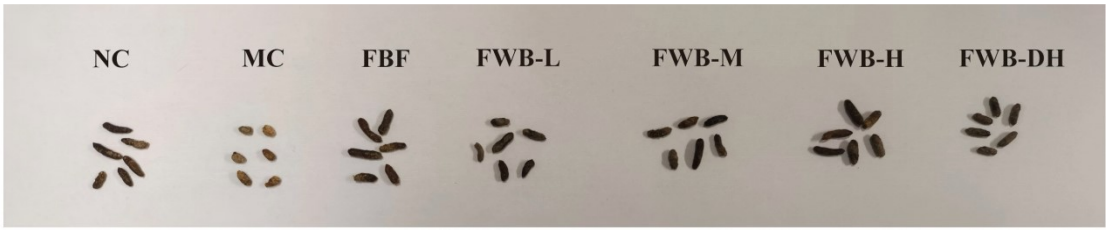



**S 1 Effect of FWB on body weight changes in loperamide-induced constipated mice**.
Body weights were monitored every three days throughout the 21-day experiment. Data are presented as mean ± SD (n=10 per group). The model control (MC) group exhibited stagnated weight gain, a typical symptom of constipation, while the normal control (NC) group showed steady growth. Treatment with Fermented Wheat By-products (FWB), particularly in the high-dose group (FWB-H), effectively alleviated this weight loss, demonstrating a growth trend approaching that of the NC group.

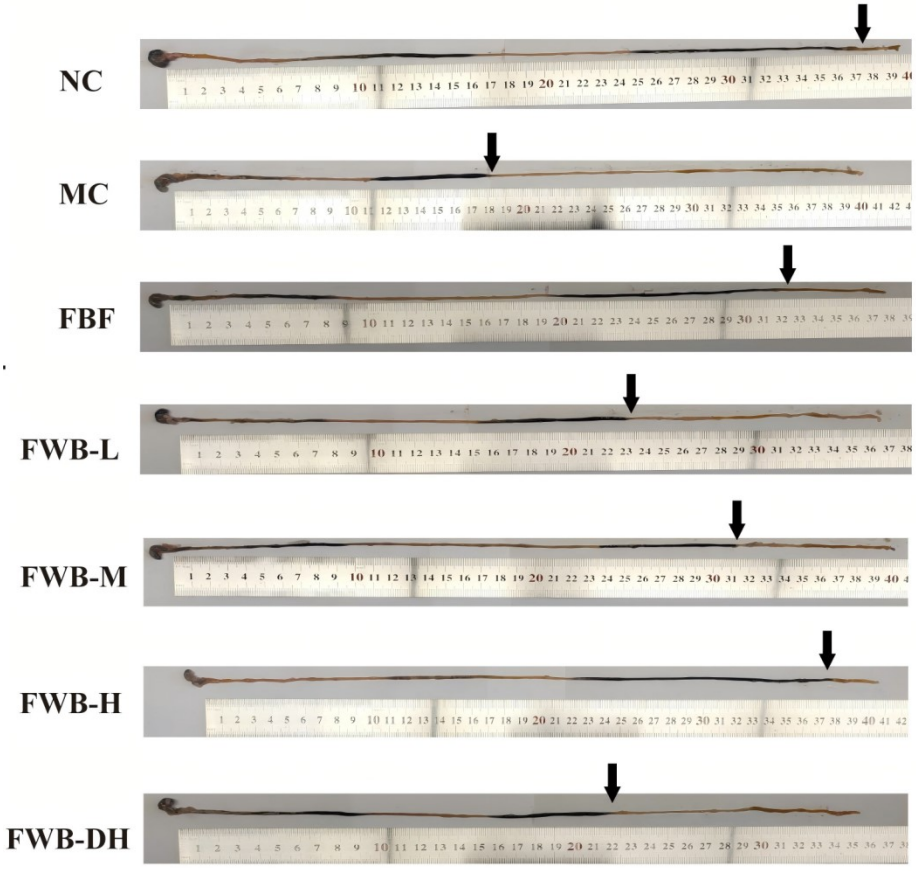



**S 2 Validation of the loperamide-induced constipation model at day 7.**
Fecal parameters were assessed before the initiation of the 21-day intervention. (A) Fecal water content (%). (B) Time to first black stool excretion (min). Data are presented as mean ± SD (n=10 per group). Statistical significance was determined by one-way ANOVA with Tukey's post hoc test. ***P < 0.001 compared with the NC group. The results confirm that all loperamide-treated

groups developed significant constipation symptoms, validating the model for the subsequent therapeutic study.

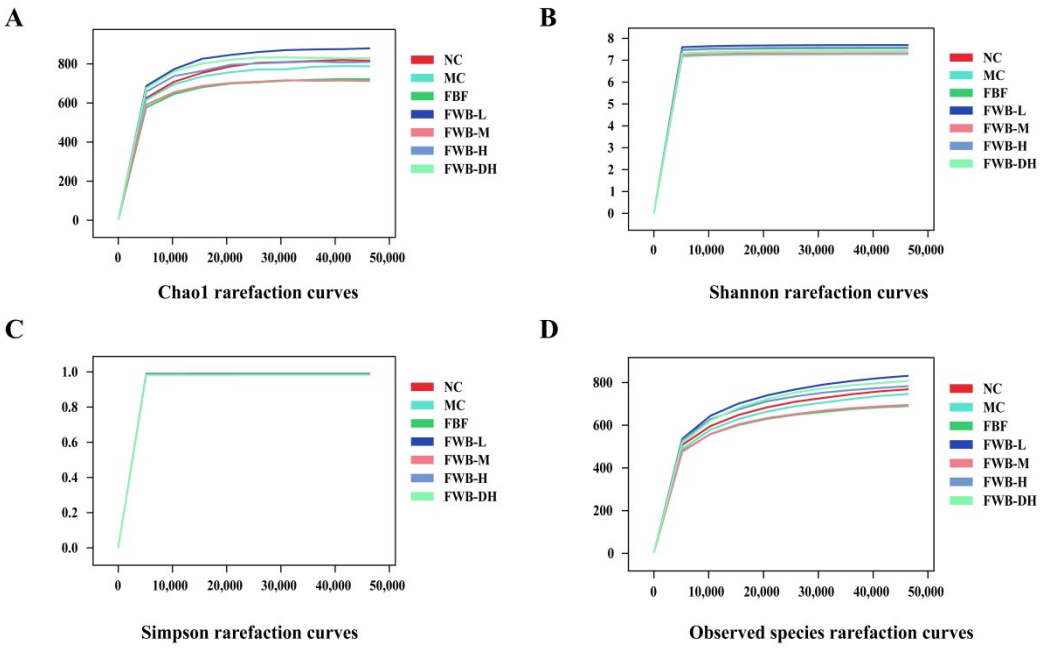



**S 3 Representative images of fecal pellet morphology after the 21-day intervention.**
The pellets from the model control (MC) group are visibly smaller, drier, and harder compared to the normal control (NC) group. Treatment with FWB, especially in the FWB-M and FWB-H groups, resulted in pellets that were larger, more moist, and more similar in appearance to the NC group, providing visual evidence of constipation alleviation.



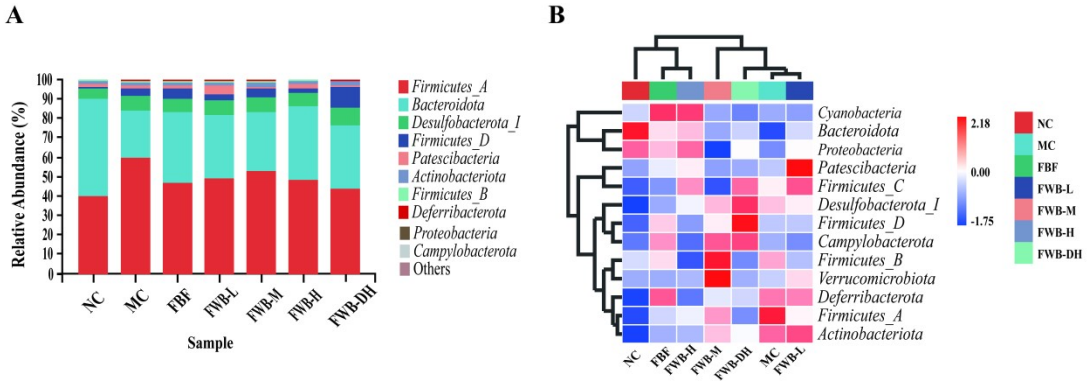

**S 4 Visual comparison of small intestinal transit across experimental groups.**

Representative images show the propulsion of activated charcoal through the small intestine, measured 30 minutes after administration on day 22. The black arrows mark the leading edge of the charcoal. The significantly shorter transit distance in the MC group reflects impaired motility. FWB treatment, in a dose-dependent manner, restored intestinal transit, with the FWB-H group showing a propulsion distance comparable to the NC group.




**S 5 Rarefaction curves assessing sequencing depth for gut microbiota analysis.**

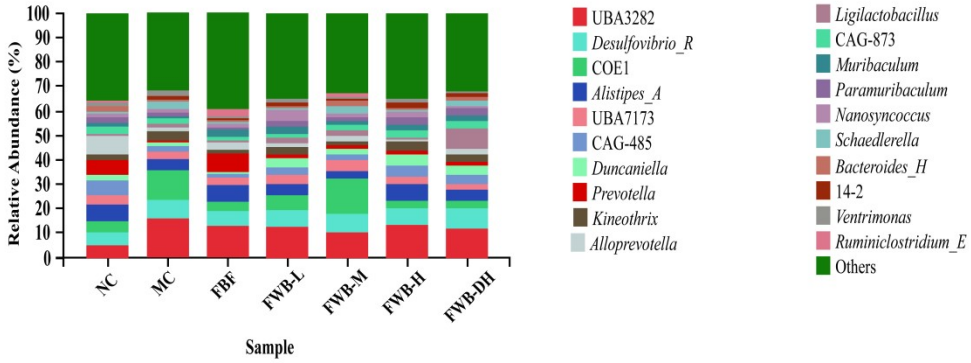
The curves represent four alpha diversity metrics: (A) Chao1 index, (B) Shannon index, (C) Simpson index, and (D) Observed species index. The flattening of all curves indicates that the sequencing depth was adequate to capture the vast majority of the microbial diversity within the samples, thus ensuring the reliability and saturation of the sequencing results.



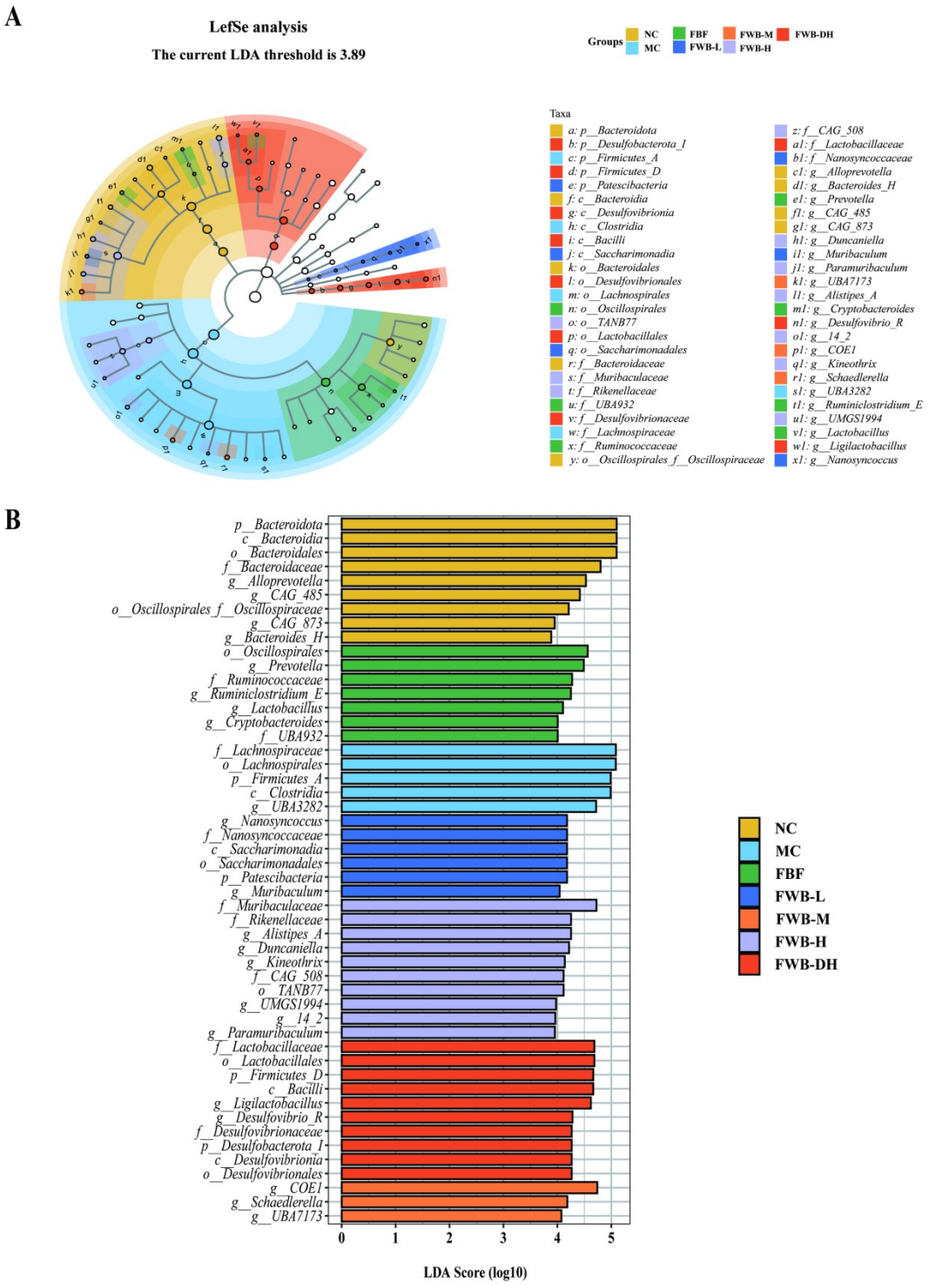

**S 6 Composition and relative abundance of intestinal microbiota at the phylum level.**

(A) Stacked bar chart displaying the relative abundance of dominant phyla across all groups. The legend indicates the different phyla. (B) Heatmap showing the relative abundance of major phyla,

clustered by treatment group. The analysis reveals that Firmicutes and Bacteroidetes are the dominant phyla. The MC group shows a characteristic increase in Firmicutes and a decrease in Bacteroidetes, which is partially reversed in the FWB-H and FBF groups, bringing their composition closer to the NC group.



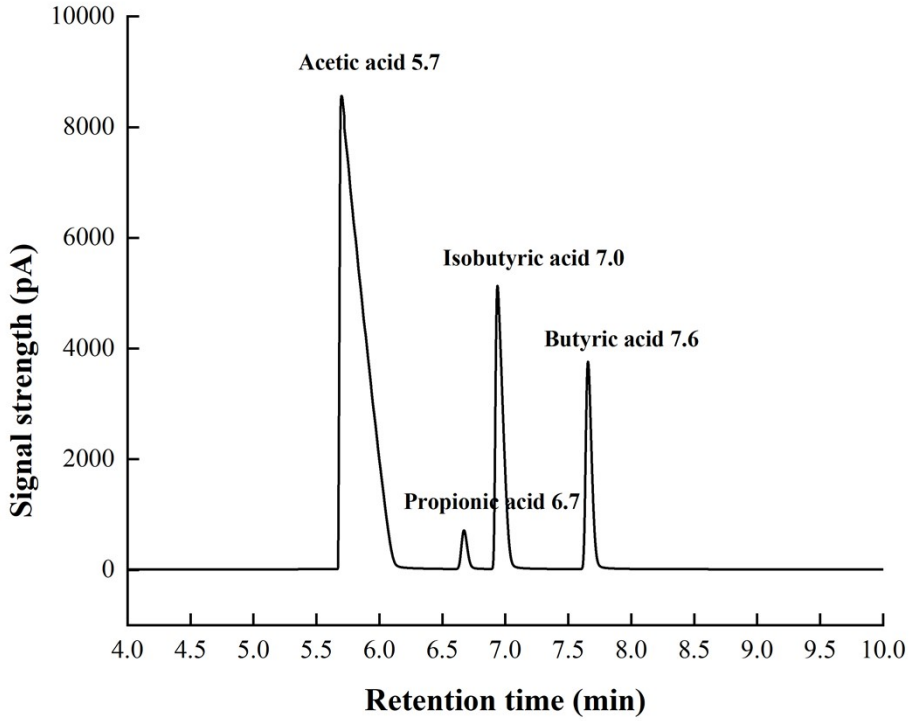

**S 7 The composition and relative abundance of intestinal microbiota at the genus level.** This stacked bar chart provides an overview of the gut microbial community structure in each group, illustrating the complex shifts that occur with constipation and FWB intervention. Key genera that are significantly altered are further analyzed and discussed in the main text and Figure S8.




**S 8 Identification of specific bacterial biomarkers using Linear discriminant analysis Effect Size (LEfSe).**

(A) A cladogram illustrating the taxonomic structure of the gut microbiota. Nodes and shadings in different colors represent the specific taxa that were significantly enriched in the

corresponding group. (B) Histogram of LDA scores computed for differentially abundant ta xa (LDA score > 3.89). This analysis highlights that the constipated (MC) group was char acterized by an enrichment of genera from the *Lachnospiraceae* family, whereas the high-d ose FWB-H group was distinguished by a significant enrichment of beneficial genera, incl uding *Duncaniella*, *Paramuribaculum*, and *Alistipes*.




**S 9 Representative gas chromatography (GC) chromatogram of short-chain fatty acids (SCFAs) in the fermented wheat by-products (FWB).**

The identified peaks and their corresponding retention times are: acetic acid (5.7 min), pro pionic acid (6.7 min), isobutyric acid (7.0 min), and butyric acid (7.6 min).

**Table S1 Box-Behnken design matrix and the response values for total SCFA production**

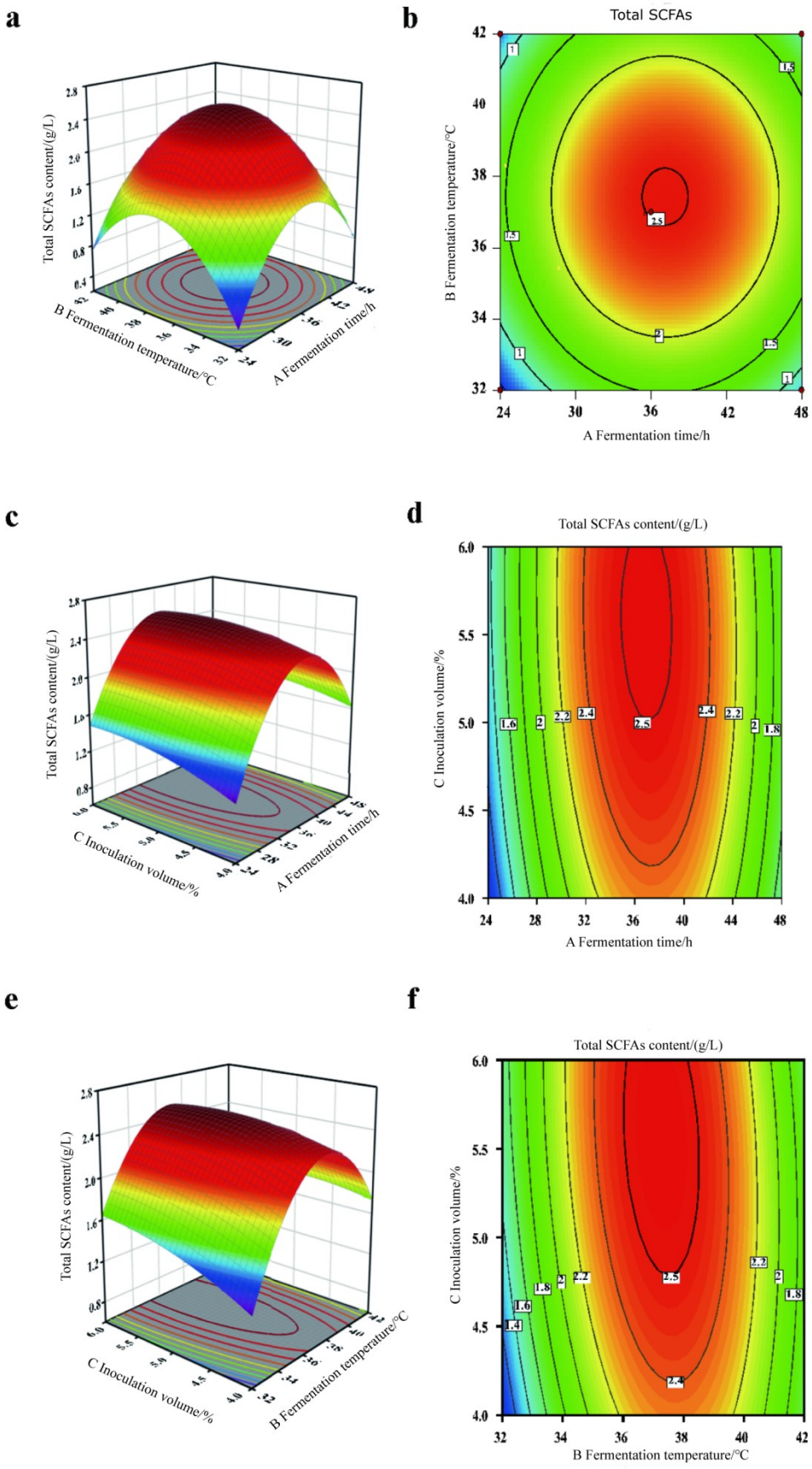
| Run | A: Fermentation time (h) | B: Temperature (°C) | C: Inoculum size (%) | Total SCFAs (g/L) |
|---|---|---|---|---|
| 1 | 48 | 32 | 5 | 0.35919 |
| 2 | 24 | 37 | 4 | 0.80089 |
| 3 | 36 | 32 | 4 | 0.85256 |
| 4 | 36 | 37 | 5 | 2.18669 |
| 5 | 36 | 37 | 5 | 2.17212 |
| 6 | 48 | 42 | 5 | 0.70677 |
| 7 | 36 | 42 | 4 | 1.30195 |
| 8 | 36 | 37 | 5 | 2.19048 |
| 9 | 36 | 32 | 6 | 1.29201 |
| 10 | 36 | 42 | 6 | 1.31889 |
| 11 | 48 | 37 | 6 | 1.34091 |
| 12 | 24 | 32 | 5 | 0.01741 |
| 13 | 48 | 37 | 4 | 1.24002 |
| 14 | 36 | 37 | 5 | 2.09273 |
| 15 | 24 | 37 | 6 | 1.07866 |
| 16 | 36 | 37 | 5 | 1.94417 |
| 17 | 24 | 42 | 5 | 0.37844 |

**Table S2 Analysis of variance (ANOVA) for the quadratic response surface model**

| Source | Sum of Squares | df | Mean Square | F-value | p-value | Significance |
|---|---|---|---|---|---|---|
| Model | 7.53 | 9 | 0.8363 | 113.95 | < 0.0001 | ** |
| A-Fermentation time | 0.1240 | 1 | 0.1240 | 16.90 | 0.0045 | ** |
| B-Fermentation temperature | 0.1717 | 1 | 0.1717 | 23.39 | 0.0019 | ** |
| C-Inoculum size | 0.0732 | 1 | 0.0732 | 9.98 | 0.0160 | * |
| AB | 0 | 1 | 0 | 0.0062 | 0.9396 | |
| AC | 0.0078 | 1 | 0.0078 | 1.07 | 0.3363 | |
| BC | 0.0446 | 1 | 0.0446 | 6.08 | 0.0431 | * |
| $A^2$ | 3.52 | 1 | 3.52 | 479.24 | < 0.0001 | ** |
| $B^2$ | 2.96 | 1 | 2.96 | 402.63 | < 0.0001 | ** |
| $C^2$ | 0.0327 | 1 | 0.0327 | 4.45 | 0.0728 | |
| Residual | 0.0514 | 7 | 0.0073 | | | |

| | | | | | |
|---|---|---|---|---|---|
| Lack of Fit | 0.0076 | 3 | 0.0025 | 0.2324 | 0.8698 |
| Pure Error | 0.0438 | 4 | 0.0109 | | |
| Cor Total | 7.58 | 16 | | | |

Note: $R^2 = 0.9932$, Adjusted $R^2 = 0.9845$. df: degrees of freedom. * $p < 0.05$ indicates significance; ** $p < 0.01$ indicates high significance.




**S 10 Response surface (3D) and contour (2D) plots illustrating the interactive effects of independent variables on the total SCFA content (g/L) during the fermentation of wheat by-**

**products.**

(a, b) The interaction between fermentation time and fermentation temperature; (c, d) The interaction between fermentation time and inoculation volume; (e, f) The interaction between fermentation temperature and inoculation volume. In each plot, the third variable was maintained at its optimal (center) level.

**Table S3 Summary of single-factor optimization for total short-chain fatty acid (SCFA) production in fermented wheat by-products (FWB)**

| Optimization Parameters | Tested Range / Variables | Optimal Condition | Max Total SCFAs (g/L) |
|---|---|---|---|
| **Medium Composition** | | | |
| Material-to-liquid ratio (g/mL) | 1:6, 1:8, 1:10, 1:12, 1:14 | 1:8 | 1.256 ± 0.04 |
| Nitrogen source | Peptone, Yeast extract, Tryptone, Ammonium sulfate | Yeast extract | 1.367 ± 0.05 |
| Yeast extract concentration (%) | 0.5, 1.0, 1.5, 2.0, 2.5 | 1.0 | 1.367 ± 0.06 |
| Inorganic salt | $FeSO_4$, $NaH_2PO_4$, $MgSO_4$, $K_2HPO_4$ | $K_2HPO_4$ | 1.818 ± 0.05 |
| $K_2HPO_4$ concentration (%) | 0.2, 0.3, 0.4, 0.5, 0.6 | 0.4 | 1.818 ± 0.04 |
| **Fermentation Conditions** | | | |
| Fermentation time (h) | 6, 12, 24, 36, 48 | 36 | 2.447 ± 0.07 |
| Temperature (°C) | 27, 32, 37, 42, 47 | 37 | 2.372 ± 0.05 |
| Inoculum size (%) | 2, 3, 4, 5, 6 | 5 | 2.218 ± 0.06 |
| Initial pH | 4.5, 5.0, 5.5, 6.0, 6.5 | 6.0 | 2.075 ± 0.04 |
| Rotation speed (rpm) | 100, 125, 150, 175, 200 | 150 | 2.141 ± 0.05 |

**Table S4 The specific SCFA composition of the fermented wheat by-products (FWB) under optimal conditions**

| SCFA Component | Concentration in FWB (g/L) | Proportion (%) |
|---|---|---|
| Acetic acid | 2.144 ± 0.05 | > 99.0% |
| Propionic acid | 0.003 ± 0.001 | < 0.2% |
| Isobutyric acid | 0.001 ± 0.000 | < 0.1% |

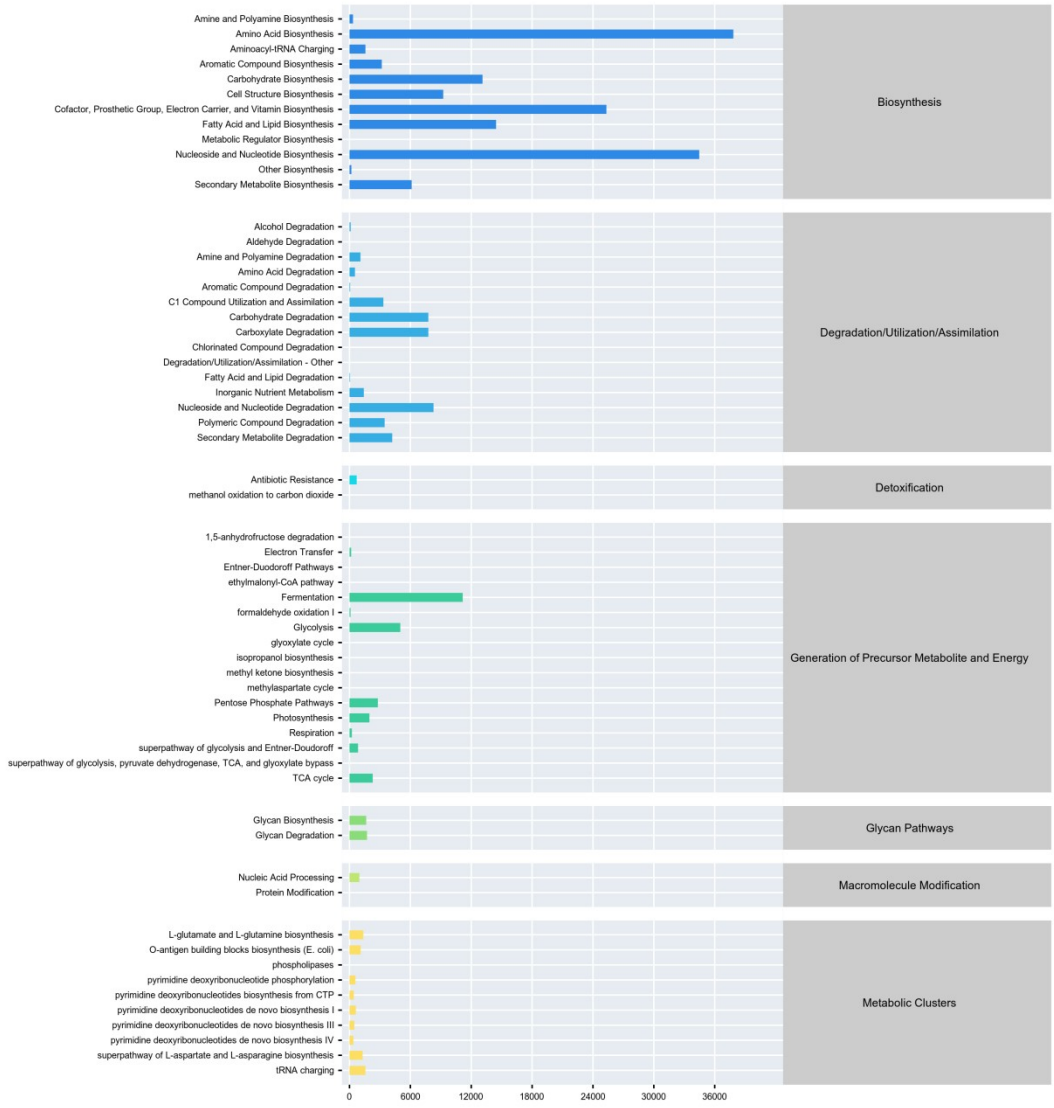
| | | |
|---|---|---|
| Butyric acid | 0.001 ± 0.000 | < 0.1% |
| Total SCFAs | 2.149 ± 0.06 | 100.0% |

**Table S5 Mean Abundance of Key Metabolic Pathways (Unit: CPM - Copies Per Million)**

| Metabolic Pathway | NC Group | MC Group | FWB-H Group | Trend (MC vs. FWB-H) |
|---|---|---|---|---|
| Pyruvate fermentation to propionate I (P108-PWY) | 756.54 | 722.38 | 877.43 | Significant increase |
| L-tryptophan biosynthesis (TRPSYN-PWY) | 1242.06 | 1294.83 | 1090.05 | Significant modulation |
| L-glutamate degradation VIII (to propionate) (P162-PWY) | 2.06 | 1.93 | 2.37 | Increase |

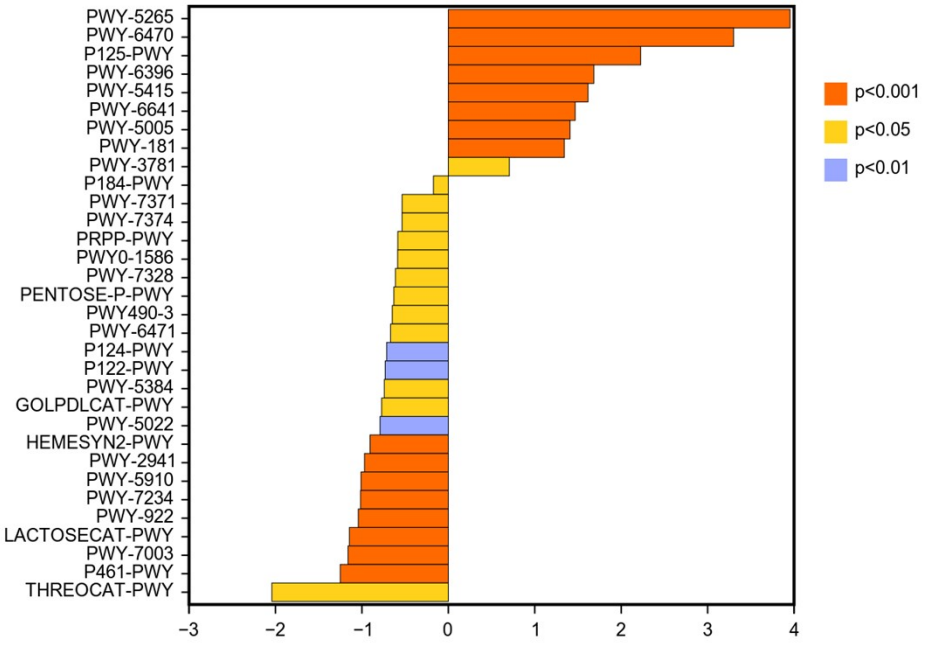
**Table S6 Abundance of Key Enzymes (EC) Related to Short-Chain Fatty Acid Synthesis**

| Enzyme Description | EC Number | NC Group | MC Group | FWB-H Group |
|---|---|---|---|---|
| Butyrate kinase | 2.7.2.7 | 12.34 | 11.31 | 12.70 |
| Phosphate butyryltransferase | 2.3.1.19 | 307.19 | 300.13 | 320.62 |
| Acetate kinase | 2.7.2.1 | 1614.59 | 1604.56 | 1611.08 |



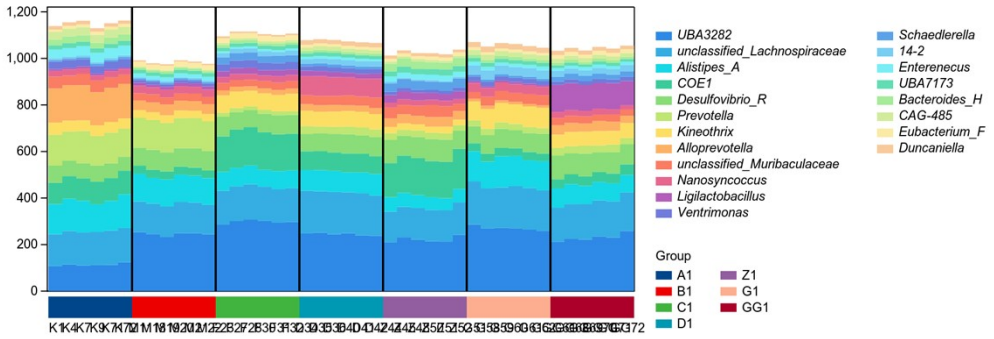

**S 11 Principal Component Analysis (PCA) of predicted metabolic pathways in gut microbiota based on MetaCyc database.**

The PCA plot illustrates the overall functional distribution of gut microbiota across different experimental groups. Each point represents an individual sample, with colors indicating different groups: NC (Normal Control, red), MC (Model Control, blue), FWB-L (Low-dose FWB, orange), FWB-M (Medium-dose FWB, green), and FWB-H (High-dose FWB, purple). PC1 and PC2 explain 18.25% and 12.83% of the total variance, respectively. A distinct separation is observed between the NC and MC groups, indicating significant functional dysbiosis induced by loperamide. Notably, a dose-dependent shift is observed in the FWB-treated groups, with the FWB-H group clustering closer to the NC group, suggesting a restorative effect of FWB on the metabolic functional potential of the microbiota.



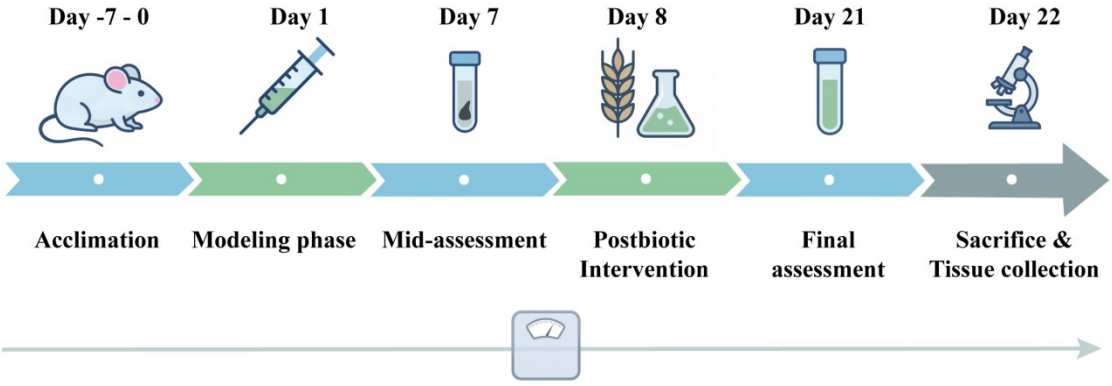

**S 12 Principal Component Analysis (PCA) of predicted metabolic pathways in gut microbiota.**

The PCA plot illustrates the overall distribution and variations in the predicted functional potential of gut microbiota across different experimental groups. Each dot represents an individual mouse sample, color-coded by group: NC (Normal Control), MC (Model Control), FWB-L (Low-dose FWB), FWB-M (Medium-dose FWB), and FWB-H (High-dose FWB). PC1 and PC2 represent the first two principal components explaining the highest proportion of the total functional variance. Distinct clustering and separation between the NC and MC groups indicate significant functional dysbiosis induced by loperamide. Notably, the FWB intervention groups, particularly the FWB-H group, exhibit a clear shift toward the NC group, suggesting that FWB effectively restores the disturbed functional metabolic profile of the microbiota.




**S 13 Principal Component Analysis (PCA) based on significantly differential predicted metabolic pathways.**

The PCA plot was constructed using only the predicted functional metabolic pathways that exhibited significant differences between experimental groups ($P < 0.05$) via PICRUSt2. Each point represents an individual sample, with colors corresponding to the experimental groups: NC (Normal Control), MC (Model Control), FWB-L (Low-dose FWB), FWB-M (Medium-dose FWB), and FWB-H (High-dose FWB). The separation between groups is more pronounced when focusing solely on these differential pathways. The MC group shifts significantly away from the NC group, while FWB intervention (particularly the FWB-H group) drives a clear restorative shift toward the NC group along the PC1 axis. This indicates that the therapeutic effects of FWB are primarily mediated through the modulation of these key differential metabolic pathways (e.g., pathways related to SCFA synthesis and tryptophan metabolism).




**S 14 Schematic timeline of the *in vivo* experimental design.**

Following a 1-week acclimation period (Days -7 to 0), mice underwent a 7-day constipation induction phase (Days 1 to 7). Subsequent postbiotic intervention was administered for 14 days (Days 8 to 21). Key mid- and final assessments for fecal parameters occurred on Days 7 and 21, respectively, followed by sacrifice and tissue collection on Day 22. Body weight and health status were continuously monitored every 3 days throughout the entire experimental period.