

Supplementary information

Heat-treated Foxtail Millet Protein Delayed the Development of Pre-diabetes to Diabetes in mice by Altering Gut Microbiota and Metabolomic Profiles

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Supplemental Figures

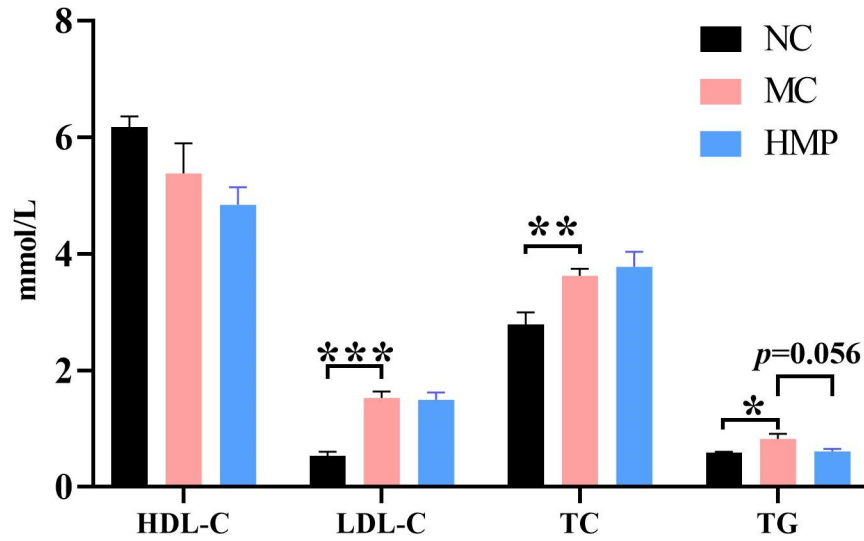


Fig. S1. Serum lipid measurements: high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) total cholesterol (TC), and triglyceride (TG). Values were analyzed by Student's t-test and expressed as the mean \pm SEM of six mice/group. Significant correlations are marked by * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. NC: normal control group; MC: model control group; HMP: heat-treated foxtail millet protein group.

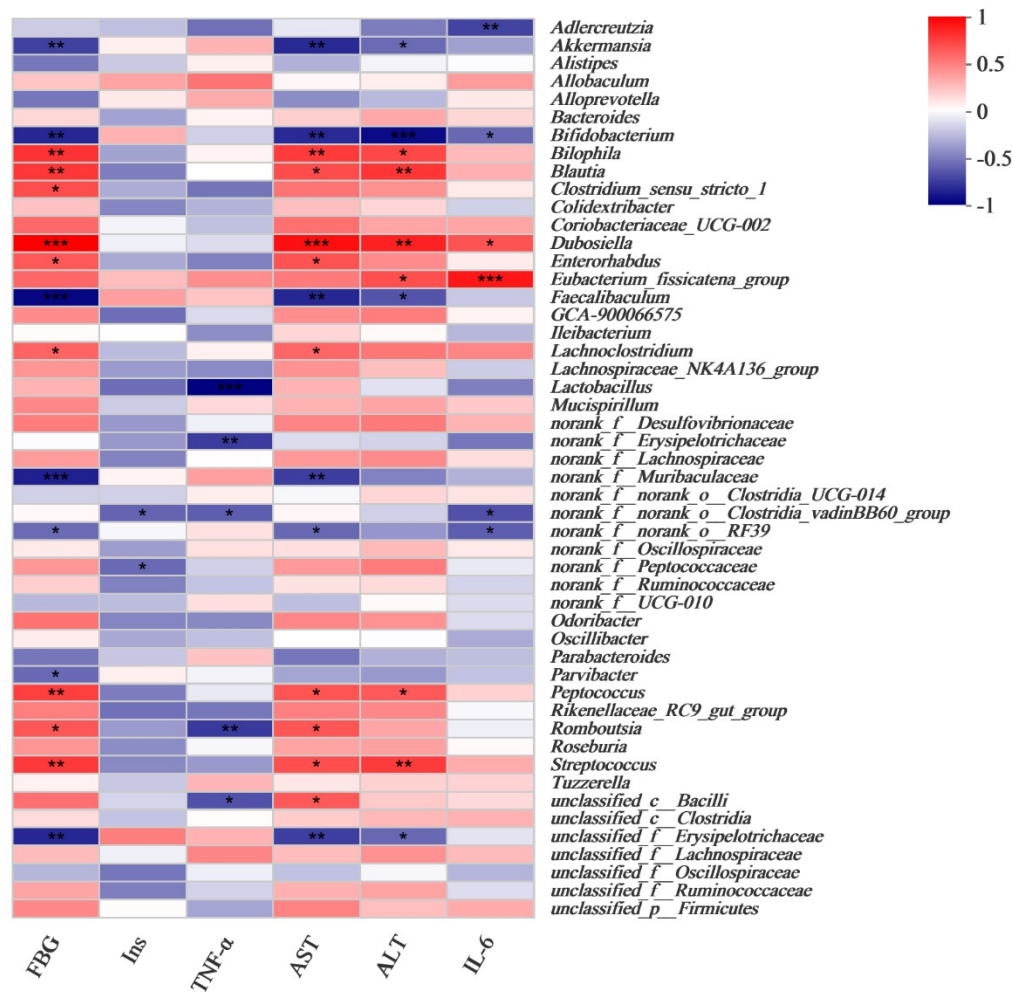


Fig. S2. The spearman correlation analysis of the associations of gut microbial changes with diabetes-related parameters. The r value is represented by the gradient color; red indicates a positive correlation, and blue color indicates a negative correlation. Means with different letters on the bar charts indicate significant differences.

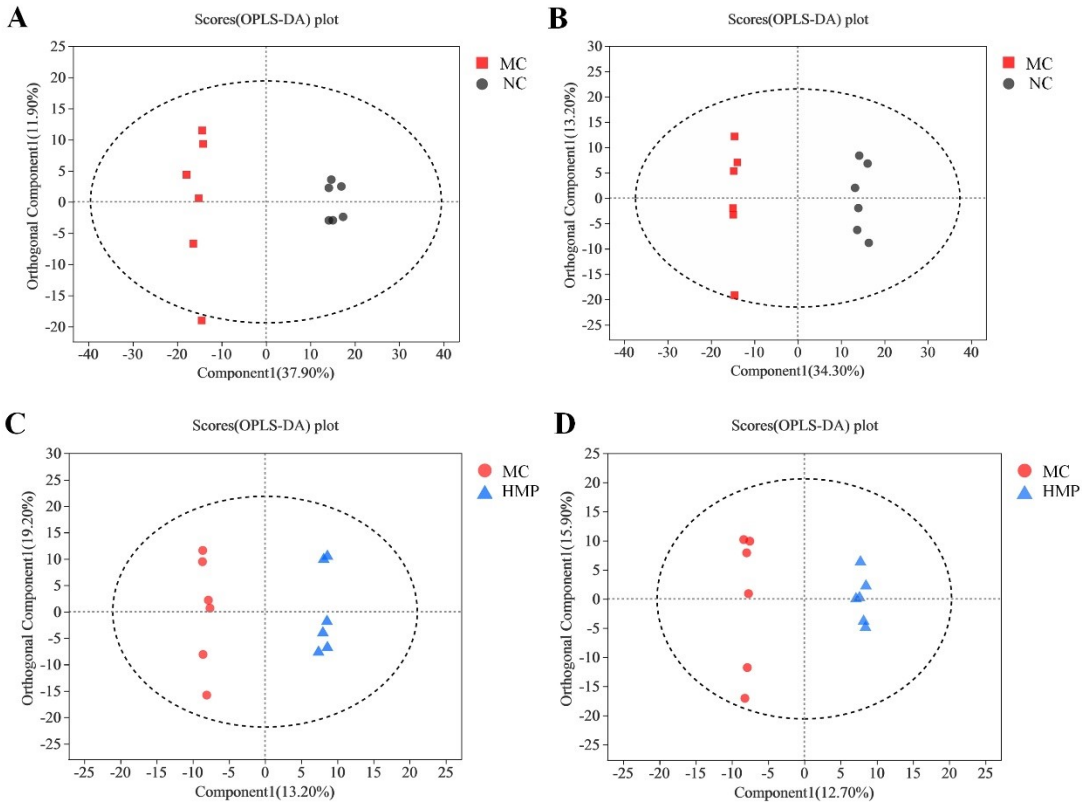


Fig. S3. Orthogonal partial least squares discrimination analysis (OPLS-DA) score plots of serum metabolic profiling in normal control (NC) vs. model control (MC) groups under positive-ion (A) and negative-ion (B) modes. MC vs. heat-treated foxtail millet protein (HMP) under positive-ion (C) and negative-ion (D).

Supplementary Tables

Tab. S1. The composition of the experimental diet

Ingredient (g/kg)	NC	MC	HMP
Heat-treated foxtail millet protein	0	0	33.6
Casein, 80 Mesh	200	200	176.48
L-Cystine	3	3	3
Corn starch	506.2	0	0
Maltodextrin 10	125	125	125
Sucrose	77.8	68.8	68.8
Cellulose, BW200	50	50	50
Soybean Oil	25	25	25
Lard	20	245	245
Mineral Mix, S10026	10	10	10
Dicalcium Phosphate	13	13	13
Calcium Carbonate	5.5	5.5	5.5
Potassium Citrate, 1 H ₂ O	16.5	16.5	16.5
Vitamin Mix, V10001	1	10	10
Choline Bitartrate	2	2	2
Source	Energy %		
Carbohydrate	70	20	20
Protein	20	20	20
Fat	10	60	60

Tab. S2. Amino acid composition of HMP

Amino acid	Content (%)
Aspartic acid/ Asp	4.65
Threonine/ Thr	3.04
Serine/ Ser	3.69
Glutamic acid/ Glu	20.93
Glycine/ Gly	1.50
Alanine/ Ala	8.56
Valine/ Val	3.64
Methionine/ Met	2.89
Isoleucine/ Ile	3.50
Leucine/ Leu	12.95
Tyrosine/ Tyr	3.18
Phenylalanine/ Phe	5.08
Lysine/ Lys	0.65
Histidine/ His	1.41
Arginine/ Arg	1.76
Proline/ Pro	5.91
Cysteine/ Gys	—

Tab. S3. Secondary structure composition of HMP

Secondary structures	Range (cm ⁻¹)	Numerical value (%)
α -Helix	1650- 1660	22.78 \pm 0.22
β -Sheet	1610- 1642	34.45 \pm 0.98
β -Turn	1660- 1680	14.22 \pm 0.58
Random coils	1642- 1650	22.56 \pm 0.01
β -Antiparallel	1680- 1770	5.99 \pm 0.18

Tab. S4. List of serum metabolites changed between HMP- MC and NC- MC groups

Metabolite	M/Z	mode	RT	FC (HMP/MC)	P_value	FC (MC/NC)	P_value
(3b,4b,11b,14b)-11-Ethoxy-3,4-epoxy-14-hydroxy-12-cyathen-15-al 14-xyloside	539.2906	neg	6.9664	1.0658	0.01602	1.1887	0.0305
(3-Nitroamino) alanine	185.9934	neg	1.0814	1.0241	0.0168	0.9578	0.003427
[2-(dimethylamino)ethoxy] sulfonic acid	150.0223	neg	0.8310	1.0253	0.02761	1.0451	0.000804
11,14,17-Eicosatrienoic acid	324.2897	pos	6.8405	0.9686	0.02066	1.0415	0.001062
13Z-Docosenamide	338.3418	pos	6.9587	0.9753	0.009463	1.0500	0.001411
1-Pyrroline-5-carboxylic acid	131.0818	pos	0.8738	1.0230	0.04955	1.0593	0.005692
20-Hydroxy-PGF2a	371.2404	pos	6.6742	0.9877	0.01773	1.0234	7.42E-05
3 alpha,7 alpha,26-Trihydroxy-5beta-cholestane	465.3586	neg	6.5448	0.9249	0.009468	1.2631	1.17E-07
3a,7a-Dihydroxy-5b-cholestan-26-al	463.3431	neg	6.8567	0.9542	0.004015	1.1361	3.95E-06
4-Hydroxy-16,18-tritriacontanedione	553.4841	neg	7.3177	1.0296	0.04464	1.0600	0.000327 4
4-Hydroxy-6-nonadecanone	321.279	pos	6.9448	0.9738	0.01811	1.0522	1.32E-05
4-Hydroxybenzenesulfonic acid	172.9907	neg	3.3777	0.9057	0.03983	0.9366	0.01818
7a,12a-Dihydroxy-cholestene-3-one	461.3273	neg	6.8226	0.9557	0.01464	1.1289	4.72E-05
7Z,10Z-Hexadecadienoic acid	503.4108	neg	7.3931	0.9766	0.03788	1.0511	0.000676 7
Acetylbalchanolide	310.2014	pos	6.1432	0.9581	2.59E-05	0.9584	0.003195
Alanyl-Lysine	452.3221	pos	6.6881	0.9896	0.0389	1.0210	0.000221
Alpha-Bisabolol oxide C	271.2269	pos	6.5425	0.9811	0.03648	1.0414	0.000299

Asperagenin	487.2798	pos	6.7919	0.9903	0.02558	1.0454	6.12E-06
Bisacurone epoxide	286.2013	pos	5.9394	0.9640	0.001034	0.9473	4.97E-05
Cibacic acid	357.2249	pos	6.6533	0.9901	0.04254	1.0232	6.03E-05
Corchoroside B	563.2905	neg	6.4725	1.0581	0.003532	1.3227	0.03111
DG (13:0/18:0/0:0)	535.4733	neg	7.8002	0.9632	0.006684	1.2972	4.50E-07
DG (14:0/0:0/18:2n6)	577.484	neg	7.2370	0.9545	0.00202	1.1897	5.85E-08
DG (14:0/15:0/0:0)	507.4418	neg	7.6051	0.9655	0.01662	1.2020	7.26E-08
DG (15:0/18:0/0:0)	581.5152	neg	7.4671	1.1005	0.004818	1.1117	0.003143
DG (15:0/18:3(6Z,9Z,12Z)/0:0)	575.4682	neg	7.2304	0.9734	0.02542	1.1150	2.77E-06
Dihydrozeatin	239.1616	pos	6.6951	0.9878	0.00484	1.0238	4.25E-06
Docosatrienoic acid	352.3211	pos	6.9378	0.9683	0.01056	1.0517	0.000195 2
Jurubine	632.3565	neg	6.4588	1.0382	0.01415	0.9492	0.000881 1
LysoPC (16:1(9Z)/0:0)	538.3153	neg	6.9596	1.0448	0.03357	0.8825	2.10E-05
LysoPC (18:1(9Z))	566.3466	neg	7.2304	1.0074	0.03073	0.9688	3.44E-06
LysoPC (20:1(11Z))	594.3779	neg	7.7201	1.0859	0.01371	0.8337	1.57E-05
Pantothenic Acid	220.1181	pos	2.9110	0.9845	0.02825	1.0489	0.000136 3
PC (17:1(9Z)/0:0)	508.3408	pos	7.0905	1.0233	0.04594	0.9415	6.87E-05
PC (18:1/0:0)	522.3558	pos	7.2495	1.0057	0.02463	0.9781	3.86E-05

PC (19:1(9Z)/0:0)	536.3718	pos	7.5768	1.0423	0.04584	0.8751	1.10E-05
PC (20:1/0:0)	572.3693	pos	7.7101	1.0416	0.009109	0.8900	2.48E-06
Propyl 2,4-decadienoate	228.196	pos	6.5355	0.9891	0.02507	1.0219	7.14E-05
Sphingosine	300.2899	pos	6.3046	0.9530	0.02797	1.0482	0.03834
Squamocin L	605.479	neg	7.2170	0.7943	0.000657	1.4804	1.42E-10
Tanacetol B	297.2035	pos	6.7021	0.9902	0.01639	1.0201	7.38E-05

Note: FC: fold change; RT: retention time; M/Z: mass-to-charge ratio; MC: model control group; NC: normal control group; HMP: heat-treated foxtail millet group. The student's t-test was applied to calculate the significance of metabolite intensities differences among different groups.

Tab. S5. Summary of the KEGG pathways influenced by HMP in mice

Pathway Description	Pathway_ID	<i>P</i> -value
Apoptosis	map04210	0.0058
Sphingolipid signaling pathway	map04071	0.0218
Necroptosis	map04217	0.0146
beta-Alanine metabolism	map00410	0.046
Pantothenate and CoA biosynthesis	map00770	0.0403
Sphingolipid metabolism	map00600	0.0361
Vitamin digestion and absorption	map04977	0.0558
Biosynthesis of unsaturated fatty acids	map01040	0.1037
Primary bile acid biosynthesis	map00120	0.0019
Choline metabolism in cancer	map05231	0.016
Glycerophospholipid metabolism	map00564	0.0738