Supplementary Information

Association between metabolic profile and microbiomic changes in rats with functional dyspepsia

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Supplementary information text:

Materials and methods

NMR data processing

All ^{1}H NMR spectra were recorded at 298 K, 600.13 MHz on Bruker AVIII 600 MHz spectrometer (Bruker Biospin, Germany). A one-dimensional pulse sequence [recycle delay-90°-t_1-90°-t_m-90°-acquisition] was used. The water signal was suppressed by irradiation during RD of 2 s, and mixing time (t_m) of 80 ms. For each sample, the 90° pulse length was adjusted to approximately 10 ms and a total of 64 transients were accumulated into 32k data points over a spectral width of 20 ppm. The ^{1}H NMR spectra were manually adjusted phase and corrected baseline using TOPSPIN (V2.1, Bruker Biospin) and referenced to the TSP resonance at d 0.00. The spectral region (d 0.50-9.50) was bucked with an equal spectra width of 0.004 ppm widths using AMIX software (V2.1, Bruker Biospin, Germany).

Data processing and statistical analysis

SIMCA-P+ (version 13.0, Umetrics, Sweden) and SPSS (V21.0, Chicago, USA) were used for statistical analyses. Data was presented as the means ± standard deviation and were processed using a two-tailed unpaired t-test with the critical *P* value set as 0.05.

The reliability of the constructed PCA, PLS-DA, OPLS-DA models was evaluated by the predicted variation Q^2 and the explained variation R^2. R^2 is defined as the proportion of variance in the data and Q^2 is defined as the proportion of variance in the data predictable. In general, R^2 and Q^2 should be larger than 0.5 to ensure that the model was well established. To further ensuring the validity of all the models, the response permutation testing was performed.

To screen metabolites and DGGE bands with striking changes that contributed to the separations between FD group and control group, OPLS-DA models were then carried out using the NMR- or DGGE-data as the X-matrix and class information as the Y-matrix with Pareto scaling. The corresponding loadings plots were generated using a MATLAB (Version 8.0, The MathWorks Inc., USA).

Supplementary tables and figures:

Table S1. ^{1}H chemical shifts for metabolites in feces on day 14

Numbers	Metabolites	δ^{1}H (ppm) and multiplicity
F1	Isocaproate	0.877(d), 1.446(m), 1.483(d), 2.199(t)
F2	Caproate	1.298(m), 1.556(m), 2.189(t)
F3	3-Methyl-2-oxovalerate	0.899(t), 1.102(d), 1.670(m), 2.925(m)
F4	Valerate	0.899(t), 1.299(m), 2.177(d)
F5	Butyrate	0.899(t), 1.568(m), 2.150(t)
F6	α-Ketoisocaproate	0.920(s), 2.051(m), 2.615(d)
F7	Isoleucine	0.948(t), 1.003(d), 1.248(m), 1.452(m), 1.989(m)
F8	Leucine	0.950(d), 0.970(d), 1.692(m), 1.718(m), 1.729(m), 3.727(m)
F9	Valine	0.993(d), 1.049(d), 2.271(m), 3.616(d)
F10	Propionate	1.061(t), 2.182(q)
F11	Methyl succinate	1.102(d), 2.129(d), 2.634(m)
F12	α-Ketoisovalerate	1.127(d), 3.021(m)
F13	N-Heptanoate	1.299(m)

F14	Lactate	1.334(d), 4.122(q)
F15	Threonine	1.335(d), 3.590(d), 4.258(m)
F16	Alanine	1.484(d), 3.781(m)
F17	Glutarate	1.775(ov), 2.180(t)
F18	Acetate	1.928(s)
F19	Glutamate	2.064(m), 2.354(m), 3.738(m)
F20	Methionine	2.142(s), 2.209(m), 2.660(t), 3.865(m)
F21	Pyruvate	2.373(s)
F22	Succinate	2.409(s)
F23	Desaminotyrosine	2.829(t), 6.875(d), 7.196(d)
F24	3-Phenylpropionate	2.495(t), 2.889(d), 7.272(t), 7.333(d), 7.373(m)
F25	Aspartate	2.697(dd), 2.831(dd), 3.906(dd)
F26	Sarcosine	2.704(s), 3.594(s)
F27	Dimethylamine	2.721(s)
F28	Asparagine	2.818(dd), 3.028(m), 3.957(dd)
F29	Trimethylamine	2.883(s)
F30	Creatine	3.020(s), 3.926(s)
F31	Tyrosine	3.053(d), 3.197(dd), 3.949(d), 6.903(dd)
F32	Malonate	3.113(s)
F33	Phenylanine	3.117(dd), 3.270(dd), 3.987(q), 7.323(d), 7.365(t), 7.418(m)
F34	Ethanolamine	3.151(d), 3.805(d)
F35	Histidine	3.144(dd), 3.249(dd), 4.001(q), 7.093(s), 7.844(s)
F36	Choline	3.203(s), 3.520(m), 4.113(m)
F37	β-Glucose	3.243(t), 3.478(m), 3.497(m), 3.864(m), 3.926(s), 4.653(d)
F38	α-Glucose	3.413(m), 3.542(m), 3.729(m), 3.740(m), 3.776(m), 3.869(m), 5.203(d)
F39	Taurine	3.243(t), 3.435(t)
F40	Methanol	3.366(s)
F41	3-Hydroxyphenylacetate	3.497(s), 6.795(m), 6.873(ov), 7.249(ov)
F42	β-Arabinose	3.525(dd), 3.672(t), 3.837(dd), 3.945(m), 4.523(d)
F43	α-Arabinose	3.855(d), 3.907(m), 3.993(t), 4.023(d), 5.241(d)
F44	Phenylacetate	3.542(s), 7.311(m), 7.375(m)
F45	Glycine	3.566(s)
F46	Serine	3.828(dd), 3.949(dd), 3.981(dd)
F47	Uracil	5.807(d), 7.542(d)
F48	Fumarate	6.525(s)
F49	Urocanate	7.317(d), 7.434(s), 7.911(s)
F50	Imidazole	7.325(s)
F51	Adenine	8.195(s), 8.213(s)
F52	Formate	8.460(s)
F53	Tauro-β-muricholate	0.694(s), 1.039(s), 1.354(m), 1.410(m), 1.731(m), 2.215(m), 2.302(m), 3.574(m)
F54	Mcthylamine	2.608(s)
F55	Trptophan	7.213(m), 7.291(m), 7.322(s), 7.544(m), 7.745(m)

F56	N-Acetylgroupcontained glytrin	2.038(s), 2.062(s)
F57	P-Hydroxyphenylacetate	3.450(s), 6.873(d), 7.184(d)
F58	Hypoxanthine	8.195(s), 8.215(s)
F59	Tauro-chenodeoxycholate acid	0.667(s)
F60	Xanthine	7.911(s)

Table S2. ^{1}H chemical shifts for metabolites in urine on day 14

Numbers	Metabolites	$\delta^{1}H$ (ppm) and multiplicity
U1	Valine	3.619(d), 2.276(m), 1.042(d), 0.983(d)
U2	Lactate	4.111(m), 1.332(d)
U3	Threonine	3.601(d), 4.261(m), 1.332(d)
U4	Alanine	3.783(dd), 1.489(d)
U5	Acetate	1.933(s)
U6	Nag C	2.042(s)
U7	Pyruvate	2.375(s)
U8	2-Ketoglutarate	2.434(t), 3.013(t)
U9	Succinate	2.418(s)
U10	Citrate	2.539(d), 2.714(d)
U11	N, N-Dimethylglycine	2.933(s), 3.682(s)
U12	Choline	3.214(s), 3.535(m), 4.086(m)
U13	PC	3.223(s), 3.603(m), 4.171(m)
U14	CPC	3.241(s), 3.693(m), 4.328(m)
U15	Taurine	3.271(t), 3.440(t)
U16	α-Glucose	5.245(d), 3.54(dd), 3.733(dd), 3.423(dd), 3.841(dd)
U17	β-Glucose	4.443(d), 3.260(dd), 3.511(dd, m), 3.403(dd, m), 3.740(dd), 3.891(dd)
U18	Glycine	3.550(s)
U19	Urea	5.790(brs)
U20	Fumarate	6.514(s)
U21	Formate	8.470(s), 3.710(m)
U22	Sarcosine	2.762(s), 3.653(s)
U23	Ethanolamine	3.141(t), 3.843(t)
U24	Creatinine	3.050(s), 4.057(s)
U25	Methylamine	2.620(s)
U26	2-Hydroxyisobutyrate	1.365(s)
U27	3-Hydroxyisovalerate	1.275(s), 2.377(s)
U28	Methylmalonate	1.243(d), 3.170(q)
U29	Succinimide	2.779(s), 5.286(s)
U30	Allantoate	5.286(s)
U31	Sucrose	5.418(d), 3.559(dd), 3.738(dd), 3.472(dd), 3.852(m), 4.220(dd, m), 4.060(d)
U32	Guanine	7.694(s)
U33	TMAO	3.268(s)

U34	Malonate	3.131(s)
U35	Guanidoacetate	3.801(s)
U36	Allothreonine	1.190(d), 4.263(m), 3.8400(d)
U37	Fucose	1.249(d), 3.808(m), 5.190(d), 3.772(m)
U38	Trigonelline	9.123(s), 8.843(dd), 8.090(d)
U39	Pantothenate	0.936(s), 0.901(s), 3.517(s), 3.402(s), 4.003(s), 2.438(t), 3.450(t)
U40	Dimethylamine	2.721(s)
U41	Allantoin	5.388(s)
U42	Cis-Aconitate	5.710(s, m), 3.120(s, d)
U43	Hippurate	7.56(dd), 7.644(t), 7.840(dd), 3.970(s), 8.548(brs)
U44	Phenylacetylglycine	3.651(s), 7.420(m), 7.360(m), 7.360(m)
U45	4-CresolGlucuronide	2.300(s), 7.230(d), 7.060(d)
U46	Methylguanidine	2.828(s)
U47	Acetamide	1.995(s)
U48	Tryptamine	7.691(dd), 7.200(td), 7.510(d), 7.280(td)
U49	Pseudouridine	7.689(s), 4.682(d), 4.292(dd), 4.162(dd)
U50	Indole-3-Acetate	7.500(d)
U51	Niacinamide	7.420(m), 8.957(d)
U52	Histidines	3.160(dd), 3.280(t), 3.970(d), 7.080(s), 7.920(s)
U53	P-Hydroxybenzoate	6.970(m), 7.770(m)
U54	Phenylalanine	3.120(dd), 3.260(d), 7.330(m), 7.350(m), 7.400(m)
U55	P-Hydroxyphenylacetate	3.460(s), 6.871(m), 7.163(m)
U56	P-Cresol	2.260(s), 6.840(d), 7.163(d)
U57	Triglycerides	0.892(m), 1.301(m), 2.043(m), 2.242(m), 2.753(m), 5.330(m)
U58	Proline	2.032(m), 2.343(m), 3.384(m), 3.401(m), 4.140(d)
U59	Creatine	3.024(s), 3.940(s)
U60	Guanidinoacetate	3.801(s)
U61	Leucine	0.920(d), 0.925(s), 1.724(m), 3.734(d)
U62	Glutamate	2.037(m), 2.338(m), 3.764(dd)
U63	N6, N6, N6-Trimethyllysine	3.110(s)
U64	Lysine	1.480(dd), 1.710(d), 1.830(m), 3.010(t)
U65	Trimethylamine (TMA)	2.892(s)
U66	Ketoleucine	0.940(d), 2.098(m), 2.618(d)
U67	N-Acetylglycoproteins	2.046(s)
U68	3-Methyl-2-Oxovalerate	0.888(t), 1.100(d), 1.424(m), 1.664(m), 2.917(m)
U69	Isobutyrate	1.053(d), 2.384(m)
U70	Isopropanol	1.143(d), 4.027(m)
U71	N-acetylglycine	2.039(s), 3.761(d)
U72	Glutamine	2.135(m), 2.443(m), 3.798(t)
U73	Acetylcarnitine	2.159(s), 2.502(m), 2.636(m), 3.201(s), 3.622(d), 3.829(dd)

Table S3 Model validation parameters on day 14

Sample	PCA		PLS-DA			OPLS-DA		
	R^2X	Q^2	R^2X	Q^2	*P*-test	R^2X	Q^2	*P*
Feces	0.873	0.602	0.733	0.979	√	0.705	0.914	0.0011
Urine	0.852	0.686	0.789	0.992	√	0.789	0.993	<0.0001

P test: permutation test, "√": model validation passed; *P* value: CV-ANOVA analysis results.

Table S4. Result of pathway analysis for urine on day 14

Pathway	Total	Expected	Hits	*P* value	Holm adjust	FDR	Impact
Citrate cycle (TCA cycle)	20	0.3994	4	0.0005	0.0402	0.0402	0.1741
Alanine, aspartate and glutamate metabolism	24	0.4793	4	0.0010	0.0823	0.0417	0.2162
D-Glutamine and D-glutamate metabolism	5	0.0999	2	0.0037	0.2929	0.1001	0
Glyoxylate and dicarboxylate metabolism	16	0.3195	2	0.0388	1	0.7865	0.4074
Butanoate metabolism	20	0.3994	2	0.0586	1	0.9487	0
Biotin metabolism	5	0.0999	1	0.0961	1	1	0
Taurine and hypotaurine metabolism	8	0.1598	1	0.1494	1	1	0.4286
Purine metabolism	68	1.3581	3	0.1509	1	1	0.0104
Methane metabolism	9	0.1797	1	0.1665	1	1	0
Nitrogen metabolism	9	0.1797	1	0.1665	1	1	0
Tyrosine metabolism	42	0.8388	2	0.2036	1	1	0
Arginine and proline metabolism	44	0.8787	2	0.2184	1	1	0
Nicotinate and nicotinamide metabolism	13	0.2596	1	0.2316	1	1	0.2381
Pantothenate and CoA biosynthesis	15	0.2996	1	0.2622	1	1	0.0204
Propanoate metabolism	20	0.3994	1	0.3339	1	1	0
Aminoacyl-tRNA biosynthesis	67	1.3381	2	0.3906	1	1	0
Valine, leucine and isoleucine degradation	38	0.7589	1	0.5402	1	1	0
Pyrimidine metabolism	41	0.8188	1	0.5680	1	1	0
Tryptophan metabolism	41	0.8188	1	0.5680	1	1	0
Primary bile acid biosynthesis	46	0.9187	1	0.6107	1.	1	0.0298

Table S5. Result of pathway analysis for feces on day 14

Pathway	Total	Expected	Hits	*P* value	Holm adjust	FDR	Impact
Methane metabolism	9	0.1091	2	0.0047	0.3841	0.3841	0
Aminoacyl-tRNA biosynthesis	67	0.8124	3	0.0439	1	0.8000	0
Purine metabolism	68	0.8245	3	0.0455	1	0.8000	0.0483
Glycerophospholipid metabolism	30	0.3638	2	0.0493	1	0.8000	0.1389
Glycine, serine and threonine metabolism	32	0.3880	2	0.0555	1	0.8000	0.2920
Linoleic acid metabolism	5	0.0606	1	0.0593	1	0.8000	0
Cyanoamino acid metabolism	6	0.0728	1	0.0707	1	0.8182	0
α-Linolenic acid metabolism	9	0.1091	1	0.1043	1	0.9384	0
Nitrogen metabolism	9	0.1091	1	0.1043	1	0.9384	0
Valine, leucine and isoleucine biosynthesis	11	0.1334	1	0.1260	1	1	0.3333
Pantothenate and CoA biosynthesis	15	0.1819	1	0.1680	1	1	0
Pyruvate metabolism	22	0.2668	1	0.2370	1	1	0.0558
Glutathione metabolism	26	0.3153	1	0.2739	1	1	0.0057
Glycolysis or Gluconeogenesis	26	0.3153	1	0.2739	1	1	0.0286
Porphyrin and chlorophyll metabolism	27	0.3274	1	0.2829	1	1	0
Arachidonic acid metabolism	36	0.4365	1	0.3591	1	1	0
Valine, leucine and isoleucine degradation	38	0.4608	1	0.3749	1	1	0
Primary bile acid biosynthesis	46	0.5578	1	0.4347	1	1	0.0298

Table S6 Result of metabolite set enrichment analysis for urine on day 14

Pathway	Total	Expected	Hits	*P* value	Holm *P*	FDR
Citric acid cycle (TCA cycle)	23	0.893	4	0.010	0.79	0.79
Glutamate metabolism	18	0.699	3	0.029	1	1
Urea cycle	20	0.777	3	0.039	1	1
Mitochondrial electron transport chain	15	0.583	2	0.112	1	1
Biotin metabolism	4	0.155	1	0.147	1	1
Ammonia recycling	18	0.699	2	0.152	1	1
Protein biosynthesis	19	0.738	2	0.166	1	1
Alanine metabolism	6	0.233	1	0.212	1	1
Taurine and hypotaurine metabolism	7	0.272	1	0.243	1	1
Malate-aspartate shuttle	8	0.311	1	0.273	1	1
Pantothenate and Coa biosynthesis	10	0.388	1	0.329	1	1
Ubiquinone biosynthesis	10	0.388	1	0.329	1	1
Aspartate metabolism	12	0.466	1	0.380	1	1
Glucose-alanine cycle	12	0.466	1	0.380	1	1
β-Alanine metabolism	13	0.505	1	0.405	1	1

Phenylalanine and tyrosine metabolism	13	0.505	1	0.405	1	1
Lysine degradation	13	0.505	1	0.405	1	1
Nicotinate and nicotinamide metabolism	13	0.505	1	0.405	1	1
Tyrosine metabolism	38	1.480	2	0.441	1	1
Propanoate metabolism	18	0.699	1	0.514	1	1
Purine metabolism	45	1.750	2	0.532	1	1
Arginine and proline metabolism	26	1.010	1	0.649	1	1
Gluconeogenesis	27	1.050	1	0.663	1	1
Tryptophan metabolism	34	1.320	1	0.747	1	1
Valine, leucine and isoleucine degradation	36	1.400	1	0.767	1	1
Pyrimidine metabolism	36	1.400	1	0.767	1	1
Bile acid biosynthesis	49	1.900	1	0.865	1	1

Table S7 Result of metabolite set enrichment analysis for feces on day 14

Pathway	Total	Expected	Hits	*P* value	Holm *P*	FDR
Protein biosynthesis	19	0.438	2	0.068	1	1
Purine metabolism	45	1.040	3	0.080	1	1
Glycine, serine and threonine metabolism	26	0.600	2	0.118	1	1
Glutathione metabolism	10	0.231	1	0.209	1	1
Aspartate metabolism	12	0.277	1	0.246	1	1
Bile acid biosynthesis	49	1.130	2	0.314	1	1
Ammonia recycling	18	0.415	1	0.346	1	1
Propanoate metabolism	18	0.415	1	0.346	1	1
Phospholipid biosynthesis	19	0.438	1	0.361	1	1
Pyruvate metabolism	20	0.461	1	0.376	1	1
Porphyrin metabolism	22	0.507	1	0.405	1	1
Methionine metabolism	24	0.553	1	0.433	1	1
Valine, leucine and isoleucine degradation	36	0.830	1	0.576	1	1

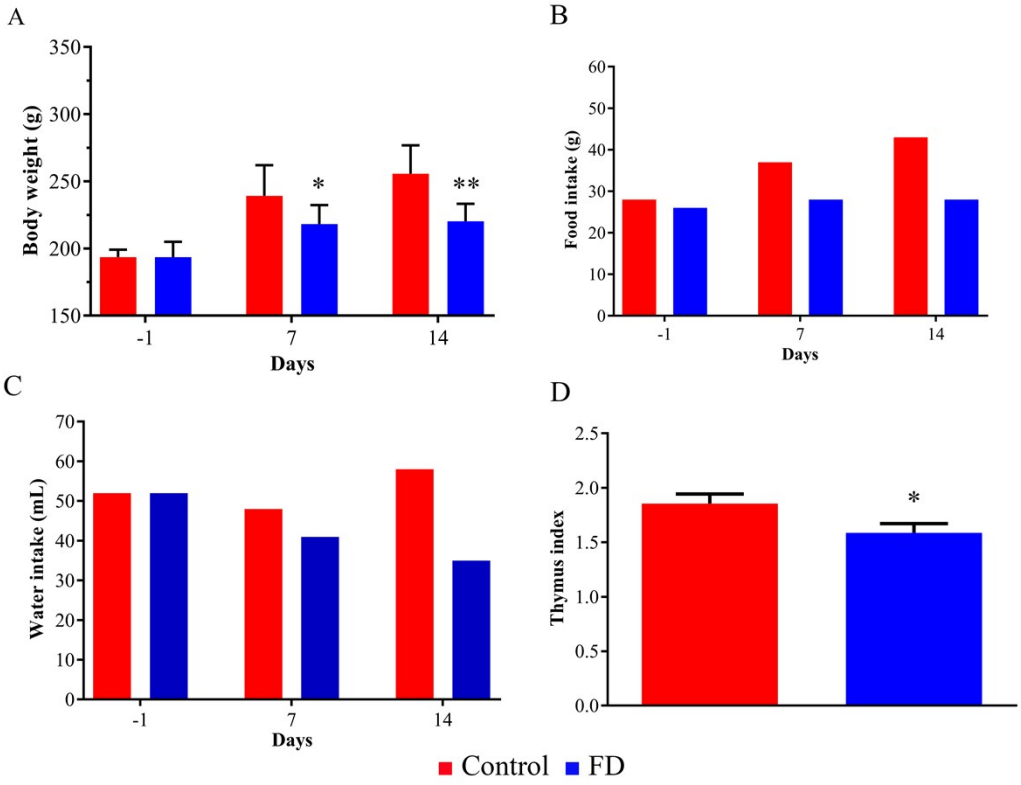

Fig. S1 The representative change of body weight (A), food intake (B), water intake (C) and thymus index (D) on day 14 in control and FD group. Value of body weight and thymus index were expressed as mean ± S.D. (n = 14) *: $P < 0.05$, **: $P < 0.01$.

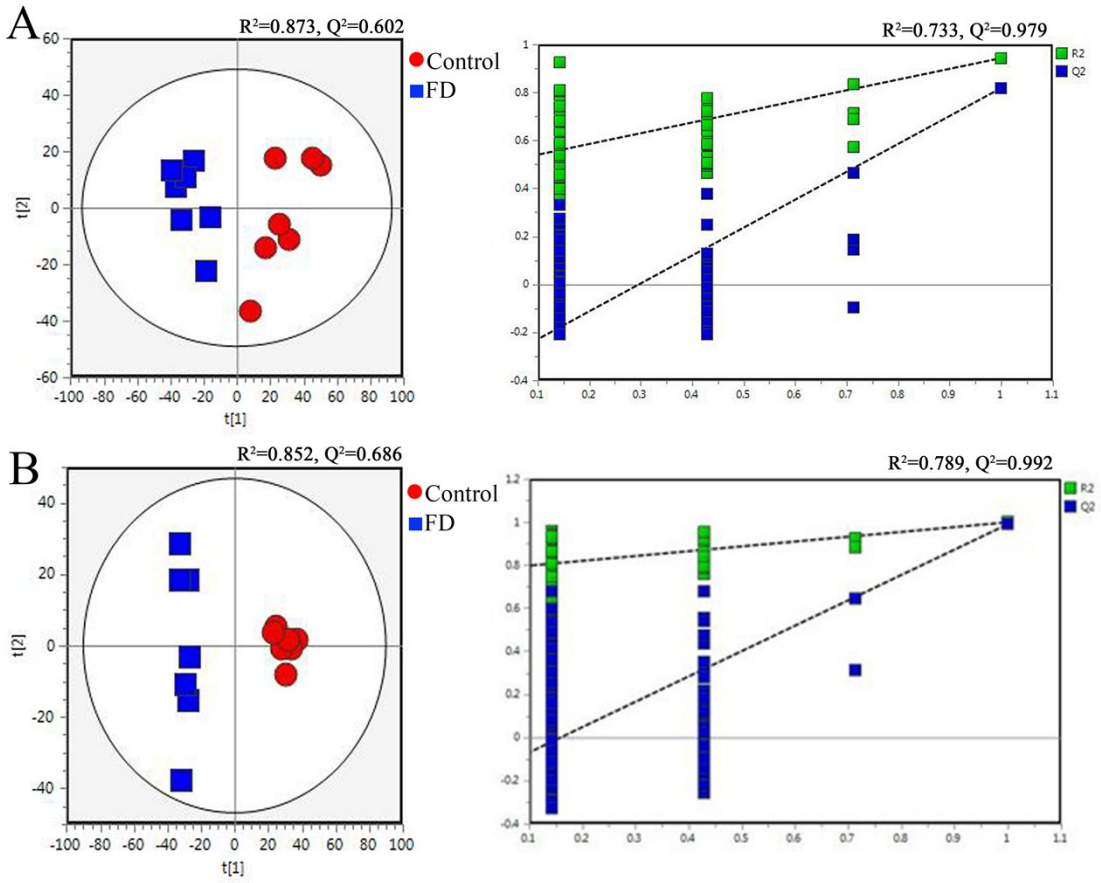

Fig. S2 PCA scores plots (left panel) and PLS-DA permutation test plots with 200 permutations (right panel) for ^{1}H NMR data of feces (A) and urine (B) on day 14. (n=14)

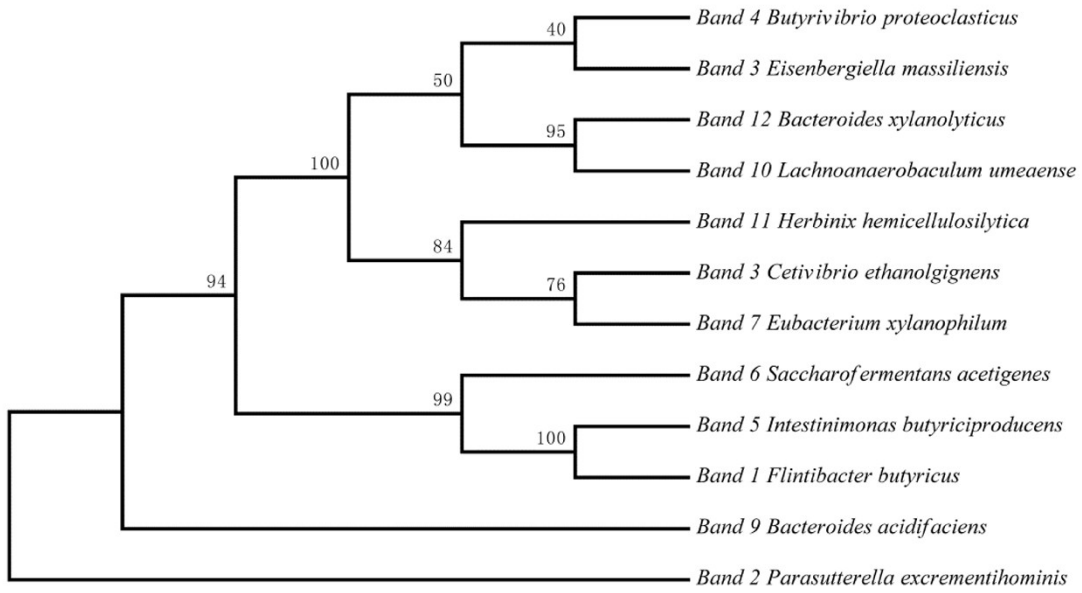

Fig. S3 Phylogenic tree of 16s rRNA V3 region sequences derived from DGGE bands.