Supplementary Information

A Holistic View into the Gallic Acid-induced Attenuation in Colitis Based on the Microbiome-Metabolomics Analysis

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Figure 1. LDA score plot

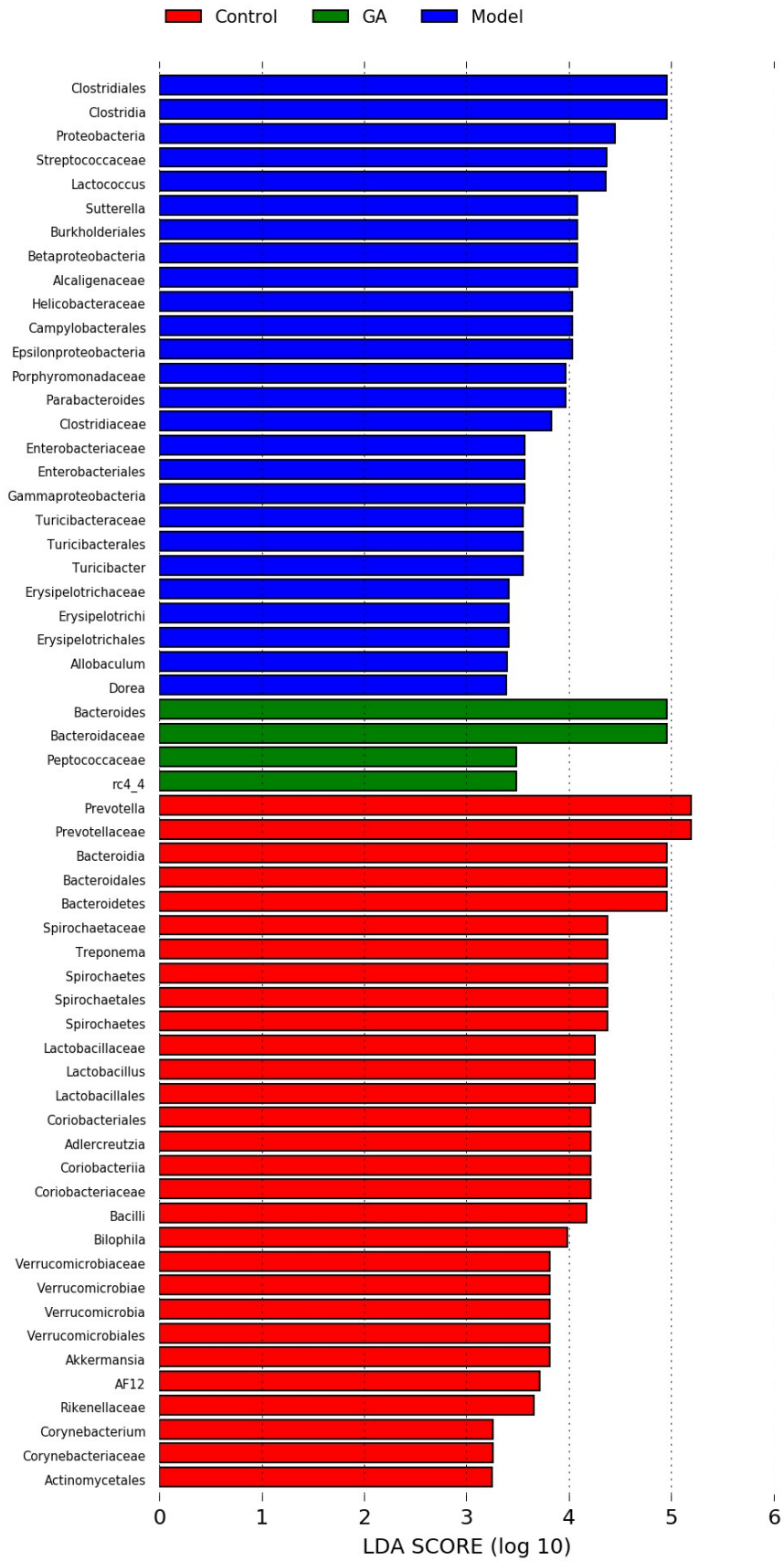
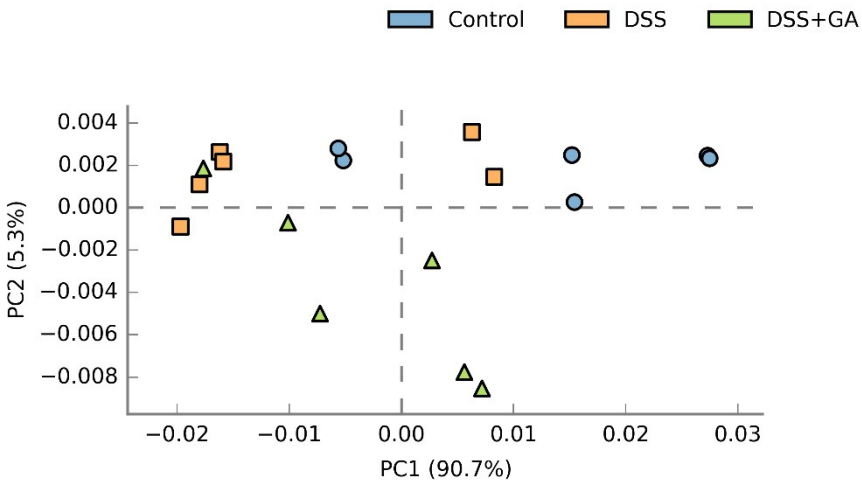


Figure 2. PCA score plot based on PICRUSt analysis



PICRUSt analysis shows the relative abundance of predicted microbial genes related to metabolism among groups based on Welch's t test.

Figure 3. PCA scores plots of 1H NMR data of feces and urine

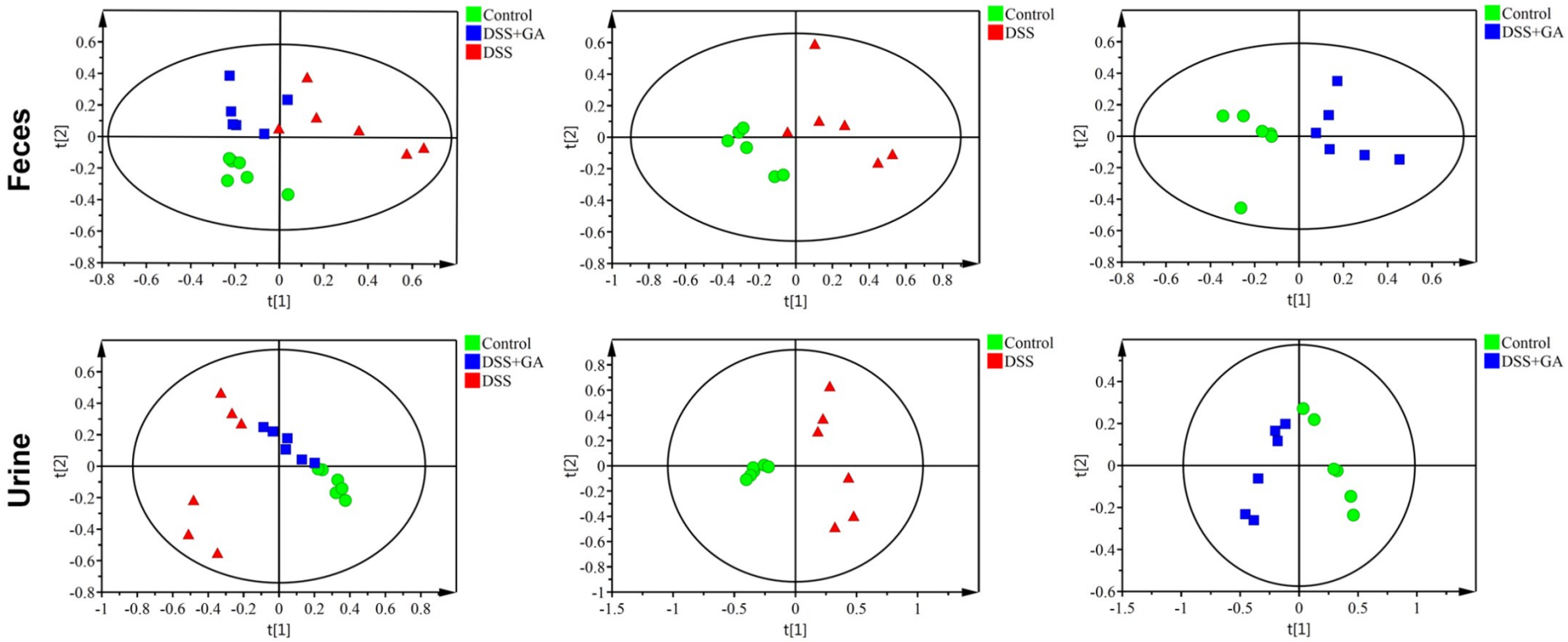


Table 1. Relative abundance of major bacterial taxa among different groups

Phylum	Family	Genus	Relative abundance(%)

			Control	DSS	DSS±GA
Actinobacteria	Corynebacteriaceae	Corynebacterium	0.19±0.17	0.00±0.00$^{\#\#}$	0.04±0.05
	Micrococcaceae	Rothia	0.01±0.00	0.01±0.01	0.01±0.01
	Bifidobacteriaceae	Bifidobacterium	0.03±0.06	0.02±0.05	0.03±0.06
	Coriobacteriaceae	Adlercreutzia	0.01±0.00	0.00±.0.00$^{\#\#}$	0.01±0.01
Bacteroidetes	Bacteroidaceae	Bacteroides	0.77±0.36	5.54±4.57$^{\#\#}$	19.70±9.77$^{*\#\#}$
	Porphyromonadaceae	Parabacteroides	0.19±0.07	2.04±2.54$^{\#\#}$	1.33±1.39$^{\#}$
	Prevotellaceae	Prevotella	31.82±9.06	3.33±5.58$^{\#\#}$	0.00±0.00$^{*\#\#}$
	Rikenellaceae	AF12	0.05±0.04	0.00±0.00$^{\#\#}$	0.00±0.00$^{\#\#}$
	S24-7	g_	25.84±3.54	21.2±3.13	25.31±13.00
	[Odoribacteraceae]	Butyricimonas	0.03±0.03	0.04±0.04	0.00±0.00$^{*\#\#}$
		Odoribacter	0.01±0.01	0.01±0.01	0.00±0.00
	[Paraprevotellaceae]	Paraprevotella	4.84±5.27	5.39±5.38	3.03±1.42
	[Paraprevotellaceae]	[Prevotella]	2.23±2.12	7.66±2.46$^{\#\#}$	9.68±8.20
Deferribacteres	Deferribacteraceae	Mucispirillum	0.01±0.01	0.01±0.01	0.00±0.00
Firmicutes	Lactobacillaceae	Lactobacillus	4.40±2.02	0.61±0.49$^{\#\#}$	2.10±1.78
	Streptococcaceae	Lactococcus	0.00±0.00	0.02±0.02$^{\#\#}$	0.00±0.00**
	Turicibacteraceae	Turicibacter	0.06±0.10	0.69±0.63$^{\#\#}$	0.13±0.13*
	Clostridiaceae	Candidatus Arthromitus	0.00±0.00	0.00±0.00	0.01±0.01
		Clostridium	0.08±0.07	0.26±0.31	0.04±0.06
	Dehalobacteriaceae	Dehalobacterium	0.04±0.02	0.02±0.02	0.10.±0.08
	Lachnospiraceae	Blautia	0.05±0.11	0.26±.32	0.10±0.14
		Coprococcus	0.20±0.32	0.35±0.16	0.57±0.44
		Dorea	0.04±0.02	0.20±0.19$^{\#\#}$	0.10±0.13
		Lachnospira	0.01±0.01	0.00±0.00	0.04±0.07

		Roseburia	0.03±0.03	0.09±0.22	0.10±0.17
		[Ruminococcus]	0.19±0.15	0.35±0.47	0.13±0.08
	Peptococcaceae	rc4-4	0.02±0.02	0.13±0.03$^{\#\#}$	0.37±0.25$^{*\#\#}$
	Ruminococcaceae	Oscillospira	2.33±1.10	5.67±4.32	5.01±2.89
		Ruminococcus	4.59±1.34	2.75±0.85$^{\#\#}$	3.50±1.63
	Veillonellaceae	Phascolarctobacterium	0.30±0.72	1.35±0.80$^{\#}$	0.98±1.39
	Erysipelotrichaceae	Allobaculum	0.01±0.00	0.44±0.35$^{\#\#}$	0.22±0.44$^{\#\#}$
		p-75-a5	0.01±0.01	0.01±0.01	0.00±0.00
Proteobacteria	Alcaligenaceae	Sutterella	0.42±0.17	2.70±1.49$^{\#\#}$	0.78±0.59*
	Desulfovibrionaceae	Bilophila	0.02±0.02	0.00±.0.00$^{\#}$	0.00±0.00$^{\#\#}$
		Desulfovibrio	0.09±0.12	0.10±0.07	0.03±0.05*
	Helicobacteraceae	Flexispira	0.24±0.24	1.09±2.03	0.50±0.47
	Enterobacteriaceae	Proteus	0.00±0.00	0.01±0.01	0.01±0.01
Spirochaetes	Spirochaetaceae	Treponema	4.18±5.25	0.26±0.42$^{\#}$	0.00±0.00$^{\#\#}$
Tenericutes	Mycoplasmataceae	Mycoplasma	0.02±0.02	0.04±0.03	0.06±0.05
Verrucomicrobia	Verrucomicrobiaceae	Akkermansia	1.22±2.68	0.00±0.00$^{\#}$	0.00±0.00$^{\#}$

The data are expressed as the mean ± SD (n=6). p vales are calculated with Mann-Whitney U test. (vs. DSS :* P<0.05,**P<0.01;vs. control :# P<0.05,##P<0.01)

Table 2. ^{1}H NMR data of metabolites in rat feces

Number	Metabolites	δ ^{1}H (ppm)
1	2-Hydroxyisobutyrate	1.35(s)
2	3-Methyl-2-oxovalerate	0.899(t), 1.093(d), 1.438(m), 1.662(m)1.685, 2.928(m), 1.924(s)
3	Alanine	1.484(d), 3.780(q)
4	Acetate	1.924(s)
5	Asparagine	2.862(dd), 2.962(m), 3.993(dd)
6	Aspartate	2.666(dd), 2.805(dd), 3.898(dd)
7	Choline	3.191(s), 3.504(m), 4.085(m)
8	Creatine	3.045(s), 3.927(s)
9	Dimethylamine	2.719(s)
10	Formate	8.460(s)
11	Fumarate	6.525(s)
12	Glycerol	3.551(dd), 3.634(m), 3.767(m) 3.768
13	Glycine	3.567(s)
14	Histidines	3.144(dd), 3.249(dd), 4.003（dd）, 7.093(s), 7.844(s)
15	Isoleucine	0.936(t), 0.993 (d), 1.249(m), 1.452(m), 1.971(m), 3.655(d)
16	Lactate	1.335(d), 4.125(q)
17	Leucine	0.925(d), 0.935(s), 1.703(m), 3.724 (d)
18	Methylamine	2.613(s)
19	Pyruvate	2.374(s)
20	Succinate	2.410(s)
21	Threonine	1.335(d), 3.578(d), 4.269(m)
22	Xanthine	7.909(s)
23	α-Glucose	3.418 (m), 3.547 (m), 3.726(m), 3.742(m), 3.772(m), 3.870 (m), 5.210(d)
24	1,3-dihydroxyacetone	3.573(s), 4.42(s)

25	2-Ketoisovalerate	1.127(d), 3.027(m)
26	3-Methyl-2-ketovalerate	0.888(t;7.4), 1.102(d;7.0), 1.461(m), 1.727(m), 2.939(m)
27	3-hydroxyphenylacetate	3.481(s), 6.757(m), 6.807(d), 6.906(dd), 7.245 (t)
28	Adenine	8.196(s), 8.213(s)
29	Butyrate	0.899(t), 1.553(m), 2.150(t)
30	Cadaverine	1.484(d), 1.727(m), 3.022(t)
31	DCA	6.859(d)
32	desaminotyrosine	3.817(d)
33	Ethanolamine	3.134(d), 7.850(d;7.6)
34	Glutarate	1.775(overlap), 2.181(t)
35	Hypoxanthine	8.196(s), 8.216(s)
36	Imidazole	7.327(s), 8.297(s)
37	Isocaproate	0.882(d), 1.449(m), 1.484 (d), 2.189 (t)
38	Malonate	3.112(s)
39	Methanol	3.366(s)
40	Methionine	2.142 (s), 2.169(m), 2.648(t), 3.853(m)
41	Methylsuccinate	1.104(d), 2.135 (d), 2.635 (m)
42	N-acetyl-glycoprotein	2.03(s)
43	NAG	2.042(s), 2.065(s)
44	Phenylacetate	3.542(s), 7.317(m), 7.375(m)
45	Propionate	1.060(t), 2.187(q), 2.374(s)
46	Sarcosine	2.705(s), 3.598(s)
47	Taurochenodeoxycholate	0.957, 0.672(s), 0.965(s)
48	TCA	0.67(s)
49	Trimethylamine	2.871(s)
50	Tyrosine	3.068(d), 3.140(dd), 3.941(d), 6.907(m), 7.196 (d/m
51	Uracil	5.807(d), 7.542(d)
52	Urocanate	6.40(d), 7.318(d), 7.433(s), 7.885(s)
53	Valerate	0.899(t), 1.309(m), 2.178(t)

54	Valine	0.972(d), 1.013(d), 2.275(m), 3.617(d), 7.909(s)
55	α-arabinose	3.849(dd), 3.906(m), 3.993(t), 4.023(d), 5.242 (d)
56	α-galactose	3.64(dd), 4.08(m), 4.58(d), 5.274 (d)
57	α-ketoisocaproate	0.920 (s), 2.054(m), 2.616(d)
58	α-ketoisovalerate	1.127(d), 3.022(m)
59	α-xylose	3.545(dd), 3.630(m), 3.670 (t), 5.203(d)
60	β-arabinose	3.525(dd), 3.670 (t), 3.856(dd), 3.945(m), 4.523(d)
61	β-L-Fucose	1.251(d：6.5), 3.452(m), 3.645 (m), 3.804 (m) , 4.561 (d;7.9)
62	β-xylose	3.23(dd), 3.46(t), 3.63(m), 3.932(dd), 4.570(d)

Abbreviation: TCA, Taurocholic acid; DCA, Deoxycholic acid; NAG, N-Acetyl group contained glycoprotein

Table 3. ^{1}H NMR data of metabolites in rat urine

1H NMR Data of Metabolites in Rat Urine		
Number	Metabolites	δ ^{1}H (ppm)
1	1-Methylnicotinamide	4.476(s),8.181(t),8.894(d),8.960(d),9.272(s)
2	2-Hydroxy-3-methylvalerate	0.8(t),0.93(d),1.15(m),1.35(m),1.75(m),3.87(d)
3	2PY	6.67(d), 7.97(dd), 8.34(d), 3.65(s)
4	3-Hydeoxyphenylacetate	3.490(s),6.794(m),6.872(m or ov),7.258(tr or ov)
5	3-Hydroxyisovalerate	1.275(s), 2.357(s)
6	4-Hydroxyhippurate	3.978(s),6.976(d),7.765(d)
7	4-hydroxyphenylacetate	6.87(d)
8	4PY	6.70(d),7.83(dd) ,8.56(d) ,3.90(s)
9	4-Pyridoxate	2.429(s),7.834(s)
10	Acetamide	1.99(s)
11	Acetoacetate	2.266(s),3.434(s)
12	Acetone	2.229(s)
13	Acetylcarnitine	2.159(s), 2.504(m), 2.631(m), 3.202(s), 3.621(d), 3.829(dd),
14	Allantoin	5.38(s), 6.02(s), 7.27(s), 8.01(s)
15	Betaine	3.273(s),3.927(s)
16	Carnitine	2.41(t),3.230(s),3.405(m),4.554(m)
17	cis-aconitate	3.123(s/d),5.711(t)
18	Creatinine	3.043(s),4.052(s)
19	Dimethyl sulfone	3.145(s)
20	Galactarate	3.95(s), 4.26(s)
21	Galactonate	4.26(s)
22	Glycerate 3-phosphate	4.22(m), 4.06(d), 3.84(d)

23	Glycolate	3.932(s)
24	Guanidoacetate	3.801(s)
25	Guanine	7.69(s)
26	Hippurate	3.975(d), 7.553(t), 7.642(t), 7.837(d), 8.508(s)
27	Hypotaurine	3.36(t)
28	Indole-3-acetate	3.681 (s), 7.161 (t), 7.216 (t), 7.507 (d), 7.635(d)
29	Isobutyrate	1.053 (d), 2.382(m)
30	Isopropanol	1.146 (d), 4.028(m)
31	Isovalerate	0.895(d), 1.929(m), 2.049(d)
32	Isovalerylglycine	0.925(d), 1.994(m), 2.166(d),3.746(d)
33	Ketoleucine	0.944 (d), 2.100 (m), 2.617(d)
34	Lactose	3.276 (t), 3.507 (m), 3.604 (m), 3.710 (m), 3.768 (m), 3.847 (m), 3.936 (m), 4.455 (d), 4.660 (d), 5.211(d)
35	Methylguanidine	2.828 (s), 3.356(s)
36	N, N-dimethylglycine	2.925 (s), 3.712(s)
37	N6, N6, N6-trimethyllysine	3.109(s)
38	N-acetylglutamate	4.13(t), 1.83(m), 2.27(t), 2.04(s)
39	N-Acetylglycine	2.044 (s), 3.763 (d), 7.997(s)
40	N-methylhydantoin	2.91(s), 4.08(s)
41	N-Nitrosodimethylamine	3.162 (s), 3.802(s)
42	N-Phenylacetylglycine	3.67(s), 3.74(d), 7.98(s)
43	O-Acetylcholine	2.143 (s), 3.202 (s), 3.711 (t/m), 4.529(m)
44	Oxypurinol	8.209(s)
45	Pantothenate	0.94(s), 0.90(s), 3.52(s), 3.40(s), 4.00(s), 3.45(t), 2.43(t)
46	p-cresol	2.253 (s), 6.817 (d), 7.130(d)
47	p-Cresol glucuronide	2.299 (s), 7.055 (m), 7.237(m)
48	p-cresol sulfate	2.347(s), 7.217 (d), 7.293(s,d,d)
49	Phenylacetylglycine	3.680 (s), 3.746 (d), 7.365 (t), 7.425(t)

50	Phosphorylcholine	3.203 (s), 3.671 (t), 4.190（dd）
51	Propylene glycol	1.14(d), 3.44(dd), 3.54(dd), 3.88(m)
52	Succinimide	2.78(s)
53	Thiamine	9.42(s), 8.04(s), 5.45(s), 3.89(t), 3.18(t), 2.56(s), 2.49(s)
54	Trans-Aconitate	3.435 (s), 6.571（s）
55	Trigonelline	4.438 (s), 8.084 (t), 8.837 (t/d), 9.122（s）
56	TMAO	3.266(s)
57	β-Glucose	3.243 (t), 3.478 (m), 3.492 (m), 3.864 (m), 3.927 (s), 4.660(d)
58	4-hydroxyphenylacetate	3.45(s), 6.87(d), 7.16(d)
59	3-hydroxyisobutyrate	1.06(d), 2.48(m), 3.52(m), 3.71(m)
60	GPC	3.24(s), 3.69(m), 4.33(m)
61	NAG	2.04(s)
62	PC	3.22(s), 3.60(m), 4.17(m)
63	Dimethylglycine	2.92(s), 3.72(s)
64	2-Hydroxyisobutyrate	1.35(s)
65	3-Methyl-2-oxovalerate	0.899(t), 1.093(d), 1.438(m), 1.662(m), 2.928(m)
66	Alanine	1.489(d), 3.780(q)
67	Acetate	1.927(s)
68	Asparagine	2.862(dd), 2.962(m), 3.995(dd)
69	Choline	3.191(s), 3.504(t/m), 4.085(m)
70	Citrulline	1.558(m), 1.865(m), 3.124(dd), 3.743(t)
71	Creatine	3.028/3.047(s), 3.926(s)
72	DMA	2.719(s)
73	Formate	8.467(s)
74	Fumarate	6.524(s)
75	Glycine	3.566(s)
76	Isoleucine	0.936(t), 0.995(d), 1.249(m), 1.452(m), 1.971(m), 3.655(d)
77	Lactate	1.331(d), 4.115(q)

78	Leucine	0.925(d), 0.936(s/d), 1.703(m), 3.725(d)
79	Pyruvate	2.373（s）
80	p-Hydroxyphenylacetate	3.445(s), 6.865 (d), 7.169(d)
81	Succinate	2.413(s)
82	Taurine	3.243(t), 3.435(t)
83	Threonine	1.330(d), 3.579(d), 4.269(m)
84	Xanthine	7.909(s)

Abbreviation: 2PY, N'-methyl-2-pyridone-5-carboxamide; 4PY, N'-methyl-4-pyridone-3-carboxamide; TMAO, Trimethylamine-N-oxide; GPC, Glycerophosphocholine; NAG, N-Acetyl group contained glycoprotein; PC, Phosphorylcholine; DMA, Dimethylamine;

Table 4. Screened metabolites with significant difference in fold change (FC) value in feces samples.

Number	Metabolites	DSS / Control			DSS+GA / Control			DSS+GA / DSS		
		FC	p value	Trend	FC	p value	Trend	FC	p value	Trend
1	Valerate	2.618	0.007	↑**				0.450	0.012	↓*
2	Butyrate	1.987	0.014	↑*				0.532	0.013	↓*
3	Glutarate	1.740	0.01	↑*				0.578	0.012	↓*
4	Taurochenodeoxycholate	1.655	0.009	↑**						
5	Glycine	1.563	0.005	↑**	1.544	0.002	↑**			
6	Methionine	1.517	0.006	↑**				0.712	0.013	↓*
7	3-Methyl-2-ketovalerate	1.515	0.015	↑*				0.640	0.013	↓*
8	Propionate	1.509	0.014	↑*				0.635	0.007	↓**
9	Methylsuccinate	1.503	0.014	↑*				0.699	0.024	↓*
10	3-Methyl-2-oxovalerate	1.465	0.029	↑*				0.627	0.013	↓*
11	Acetate	1.465	0.027	↑*				0.688	0.030	↓*
12	Dimethylamine	0.809	0.002	↓**						
13	Adenine	0.800	0.046	↓*						
14	Uracil	0.776	0.025	↓*						
15	Fumarate	0.739	<0.001	↓***	0.733	0.003	↓**			
16	Deoxycholic acid	0.637	<0.001	↓***	0.703	0.002	↓**			
17	Asparagine				1.264	<0.001	↑***			
18	Desaminotyrosine				1.261	0.005	↑**	1.212	0.032	↓*
19	Alpha-arabinose				1.208	0.005	↑**			
20	Xanthine				0.750	<0.001	↓***			
21	α-galactose							1.234	<0.001	↑***
22	β-xylose							1.280	0.003	↑**
23	β-arabinose							1.249	0.004	↑**
24	α-xylose							1.240	0.008	↑**

25	Isocaproate	□	□	□	□	□	□	□	□	0.782	0.018	↓*

*p<0.05; **p<0.01, ***p<0.001

Table 5. Screened metabolites with significant difference in fold change (FC) value in urine samples

Number	Metabolites	DSS / Control			DSS+GA / Control			DSS+GA / DSS		
		FC	p value	Trend	FC	p value	Trend	FC	p value	Trend
1	Oxypurinol	0.337	<0.001	↓***				2.771	<0.001	↑***
2	Xanthine	0.588	<0.001	↓***				1.691	<0.001	↑***
3	4-Hydroxyhippurate	0.714	<0.001	↓***	0.648	<0.001	↓***			
4	Hippurate	0.719	<0.001	↓***	0.650	<0.001	↓***			
5	cis-aconitate	0.792	<0.001	↓***						
6	Galactarate	0.815	<0.001	↓***						
7	Isovalerate	1.421	<0.001	↑***	1.280	<0.001	↑***			
8	Acetoacetate	0.723	0.045	↓*						
9	Succinate	1.605	0.021	↑*						
10	Fumarate	0.806	0.020	↓*						
11	Isopropanol	0.707	0.016	↓*	0.742	0.033	↓**			
12	Acetate	2.154	0.011	↑*	1.316	0.002	↑**			
13	Taurine	0.646	0.008	↓**						
14	trans-Aconitate	0.506	0.008	↓**						
15	Trigonelline	0.830	0.006	↓**				1.219	0.002	↑**
16	4-Pyridoxate	0.741	0.005	↓**	0.628	<0.001	↓***			
17	2-Hydroxyisobutyrate	1.542	0.003	↑**	1.338	<0.001	↑***			
18	Formate	1.659	0.001	↑**				0.661	0.010	↓**
19	Indole-3-acetate				0.674	<0.001	↓***			
20	succinimide				1.239	<0.001	↑***			
21	p-Cresol glucuronide				0.753	<0.001	↓***			
22	Glycolate				0.783	<0.001	↓***			
23	Asparagine				0.800	<0.001	↓***			
24	Phenylacetylglycine				0.797	<0.001	↓***			

*p<0.05; **p<0.01, ***p<0.001