

Table 1 The composition of animal diets

Composition	C	M	I
Feed (%)			
Crude protein	30.0	30.0	30.0
Crude fat	4.0	4.0	4.0
Soluble carbohydrate	40.0	40.0	32.5
Crude fiber	5.0	5.0	12.5
Calcium	1.8	1.8	1.8
Phosphorus	1.2	1.2	1.2
Moisture	10.0	10.0	10.0
Ash content	10.0	10.0	10.0
Water		1.0% choline	1.0% choline

Note: C, M and I represent the control group, model group and dietary fiber intervention group, respectively. The M and I group were given with 1.0% choline supplemented in drinking water.

Table 2 The daily intake per mouse for the diets

	C	M	I
Feed (g)			
Crude protein	0.96	1.14	0.90
Crude fat	0.13	0.15	0.12
Soluble carbohydrate	1.28	1.52	0.98
Crude fiber	0.16	0.19	0.38
Calcium	0.06	0.07	0.05
Phosphorus	0.04	0.05	0.04
Moisture	0.32	0.38	0.30
Ash content	0.26	0.30	0.24
Water (mL)	23.3	25.2	24.8
Choline (mg)	-	252	248

Note: C, M and I represent the control group, model group and dietary fiber intervention group, respectively. Data are presented as the mean values (n =10). The food intake was estimated by assuming from the diet composition and logs database.

Table 3 The Operational Taxonomic Units (OUTs) relative abundances for groups at phylum level

Phylum	C	M	I
Bacteroidetes	37.88%	21.18%	54.86%
Firmicutes	30.52%	51.44%	16.82%
Verrucomicrobia	2.12%	1.55%	4.43%
Chlamydiae	0.48%	1.90%	0.39%
Proteobacteria	1.91%	2.21%	1.46%
Actinobacteria	8.10%	4.90%	1.91%
Candidatus Saccharibacteria	0.14%	0.10%	0.09%
Fibrobacteres	0.03%	0.02%	0.05%
Spirochaetes	0.03%	0.03%	0.03%
Tenericutes	0.02%	0.04%	0.01%
Others	18.78%	16.65%	19.97%

Table 4 The top 35 genera were annotated according to the species and abundance information of samples identified in mice fed the defined diets

genus	C	M	I	Tax_detail
<i>Lactobacillus</i>	10.50%	11.73%	1.38%	p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;
<i>Bacteroides</i>	10.44%	6.71%	14.78%	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;
<i>Prevotella</i>	7.96%	3.63%	10.63%	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;
<i>Clostridium</i>	1.85%	2.26%	0.95%	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae
<i>Dubosiella</i>	0.47%	2.20%	0.42%	p__Firmicutes;c__Erysipelotrichia;o__Erysipelotrichales;f__Erysipelotrichaceae;
<i>Bifidobacterium</i>	1.96%	0.85%	4.49%	p__Actinobacteria;c__unidentified_Actinobacteria;o__Bifidobacteriales;
<i>Paenarthrobacter</i>	0.47%	1.89%	0.39%	p__Chlamydiae;c__Chlamydiia;o__Chlamydiales;f__Chlamydiaceae;
<i>Akkermansia</i>	2.10%	1.53%	4.39%	p__Verrucomicrobia;c__Verrucomicrobiae;o__Verrucomicrobiales;
<i>Enterorhabdus</i>	0.58%	1.50%	0.28%	p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Eggerthellaceae;
<i>Eubacterium</i>	0.60%	0.27%	1.15%	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Eubacteriaceae;
<i>Desulfovibrio</i>	0.38%	0.84%	0.38%	p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;
<i>Alistipes</i>	1.11%	0.80%	1.99%	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;
<i>Ruminococcus</i>	0.67%	0.24%	0.67%	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;
<i>Pseudoflavonifractor</i>	0.50%	0.76%	0.61%	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Unclassified;
<i>Roseburia</i>	0.45%	0.14%	0.59%	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;
<i>Parabacteroides</i>	1.08%	0.59%	1.59%	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Tannerellaceae;
<i>Faecalibaculum</i>	0.12%	0.52%	0.06%	p__Firmicutes;c__Erysipelotrichia;o__Erysipelotrichales;f__Erysipelotrichaceae;
<i>Lachnoclostridium</i>	0.29%	0.36%	0.24%	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;
<i>Oscillibacter</i>	0.26%	0.32%	0.39%	p__Firmicutes;c__Clostridia;o__Clostridiales;
<i>Flavonifractor</i>	0.26%	0.31%	0.30%	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Unclassified;
<i>Dorea</i>	0.18%	0.31%	0.19%	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;
<i>Turcibacter</i>	0.29%	0.51%	0.01%	p__Firmicutes;c__Erysipelotrichia;o__Erysipelotrichales;f__Erysipelotrichaceae;
<i>Anaerotruncus</i>	0.11%	0.25%	0.13%	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;
<i>Adlercreutzia</i>	0.30%	0.18%	0.13%	p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;
<i>Blautia</i>	0.11%	0.17%	0.09%	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;
<i>Barnesiella</i>	0.27%	0.15%	0.40%	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Barnesiellaceae;
<i>Butyrivibrio</i>	0.18%	0.12%	0.86%	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;
<i>Faecalibacterium</i>	0.07%	0.17%	0.12%	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;
<i>Butyricimonas</i>	0.18%	0.11%	0.37%	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Odoribacteraceae;
<i>Rikenella</i>	0.19%	0.09%	0.86%	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;
<i>Odoribacter</i>	0.19%	0.04%	0.92%	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;
<i>Olsenella</i>	0.12%	0.04%	0.62%	p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Atopobiaceae;
<i>Millionella</i>	0.06%	0.03%	0.26%	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;
<i>Libanicoccus</i>	0.19%	0.01%	0.03%	p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Atopobiaceae;
<i>Helicobacter</i>	0.51%	1.10%	0.00%	p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Helicobacteraceae;

Table 5 The Spearman correlation analysis between species and the key physicochemical parameters of choline metabolism

	<i>r</i> (TMA)	<i>p</i> value	<i>r</i> (TMAO)	<i>p</i> value
<i>Lactobacillus</i>	0.504	0.257	0.524	0.246
<i>Bacteroides</i>	-0.692	0.039	-0.684	0.042
<i>Prevotella</i>	-0.539	0.134	-0.529	0.143
<i>Clostridium</i>	0.383	0.332	0.4	0.321
<i>Dubosiella</i>	0.892	0.001	0.887	0.003
<i>Bifidobacterium</i>	-0.623	0.028	-0.606	0.017
<i>Paenarthrobacter</i>	0.884	0.102	0.88	0.112
<i>Akkermansia</i>	-0.913	0.005	-0.938	0.003
<i>Enterorhabdus</i>	0.686	0.142	0.678	0.145
<i>Eubacterium</i>	-0.64	0.063	0.632	0.060
<i>Desulfovibrio</i>	0.814	0.008	0.809	0.012
<i>Alistipes</i>	-0.453	0.288	-0.471	0.277
<i>Ruminococcus</i>	-0.856	0.053	-0.852	0.054
<i>Pseudoflavonifractor</i>	0.676	0.046	0.679	0.044
<i>Roseburia</i>	-0.395	0.293	-0.386	0.304
<i>Parabacteroides</i>	-0.418	0.263	-0.408	0.276
<i>Faecalibaculum</i>	0.816	0.107	0.812	0.108
<i>Lachnoclostridium</i>	0.606	0.200	0.61	0.198
<i>Oscillibacter</i>	-0.599	0.202	-0.599	0.201
<i>Flavonifractor</i>	0.797	0.203	0.779	0.113
<i>Dorea</i>	0.506	0.256	0.508	0.225
<i>Turicibacter</i>	0.671	0.814	0.665	0.504
<i>Anaerotruncus</i>	0.453	0.221	0.45	0.224
<i>Adlercreutzia</i>	-0.614	0.195	-0.607	0.211
<i>Blautia</i>	0.401	0.285	0.399	0.288
<i>Barnesiella</i>	-0.441	0.235	-0.445	0.292
<i>Butyrivibrio</i>	-0.615	0.195	-0.636	0.184
<i>Faecalibacterium</i>	0.379	0.153	0.378	0.136
<i>Butyricimonas</i>	-0.504	0.257	-0.513	0.252
<i>Rikenella</i>	-0.445	0.145	-0.552	0.131
<i>Odoribacter</i>	-0.511	0.253	-0.53	0.242
<i>Olsenella</i>	-0.61	0.198	-0.63	0.187
<i>Millionella</i>	-0.373	0.338	-0.384	0.331
<i>Libanicoccus</i>	-0.639	0.064	-0.643	0.062
<i>Helicobacter</i>	0.465	0.205	0.455	0.219

Note: Data are mean. *r* represents the correlation coefficient of environmental factors and species distribution, where the larger the value, the greater biological relevance between environmental factors and species distribution. *p* value < 0.05 means the correlation is significant at the 0.05 level (2-tailed). *p* value < 0.01 means the correlation is highly significant at the 0.01 level (2-tailed).

Table 6 Relative abundance (%) of gene clusters responsible for choline metabolism and TMA lyase activity

Group	1.97.1.- (CutD)	4.3.99.4 (CutC)	TMA lyase
C	0.000013±0.000001	0.000013±0.000001	0.06±0.03
CG	0.000016±0.000000	0.000016±0.000000	0.10±0.03
CGM	0.000020±0.000001	0.000020±0.000001	0.16±0.02
M	0.000050±0.000001	0.000060±0.000002	0.48±0.06
MG	0.000060±0.000001	0.000070±0.000001	0.66±0.06
MGM	0.000067±0.000001	0.000078±0.000001	0.99±0.07
I	0.000030±0.000001	0.000040±0.000001	0.24±0.04
IG	0.000033±0.000001	0.000033±0.000001	0.33±0.04
IGM	0.000036±0.000001	0.000036±0.000001	0.44±0.05

Note: C(M, I), C (M, IG), and C(M, I)GM, represent anaerobic culture of intestinal digesta samples removed from the mice fed on the C(M, I) group diet using a medium supplied with 1.0% choline, a mixture of 1.0% glycine and 1.0% choline, another of 1.0% glycine, 1.5 mmol L⁻¹ methylglyoxal and 1.0% choline.

Table 7 The relative abundance of enzymes associated with biosynthesis and utilization of glycine

KEGG_Description	EC	ID	C	CG	CGM	M	MG	MGM	I	IG	IGM
Glycine, serine and threonine metabolism											
choline dehydrogenase	1.1.1.1	<i>gbsB</i>	0.000063	0.000056	0.000052	0.000118	0.000110	0.000101	0.000024	0.000020	0.000018
betaine-homocysteine S-methyltransferase	2.1.1.5	<i>BHMT</i>	0	0	0	0.000003	0.000002	0.000001	0	0	0
sarcosine oxidase / L-pipecolate oxidase	1.5.3.1	<i>PIPOX</i>	0.000002	0.000002	0.000001	0.000008	0.000006	0.000005	0.000005	0.000003	0.000002
glycine N-methyltransferase	2.1.1.20	<i>GNMT</i>	0	0	0	0.000001	0.000001	0	0	0	0
threonine aldolase	4.1.2.48	<i>ItaE</i>	0.000152	0.000160	0.000147	0.000113	0.000136	0.000102	0.000224	0.000236	0.000215
threonine 3-dehydrogenase	1.1.1.103	<i>TDH</i>	0.000014	0.000018	0.000001	0.000019	0.000028	0.000009	0.000020	0.000023	0.000015
glycine C-acetyltransferase	2.3.1.29	<i>kbl</i>	0.000087	0.000093	0.000081	0.000055	0.000068	0.000041	0.000150	0.000158	0.000142
monoamine oxidase	1.4.3.4	<i>aofH</i>	0.000001	0.000003	0	0	0	0	0.000001	0.000003	0.000001
glycine hydroxymethyltransferase	2.1.2.1	<i>glyA</i>	0.000300	0.000291	0.000288	0.000361	0.000353	0.000348	0.000259	0.000250	0.000243
L-serine/L-threonine ammonia-lyase	4.3.1.19	<i>SDS</i>	0.000248	0.000240	0.000256	0.000228	0.000223	0.000233	0.000193	0.000187	0.000196
L-serine dehydratase	4.3.1.17	<i>sdaA</i>	0.000255	0.000241	0.000263	0.000321	0.000310	0.000328	0.000232	0.000219	0.000241
Glycolysis/Gluconeogenesis											
sugar PTS system EIIA component	2.7.1.-	<i>crr</i>	0.000457	0.000454	0.000452	0.000996	0.000991	0.000989	0.000219	0.000217	0.000215
glucose PTS system EIIB component	2.7.1.199	<i>ptsGb</i>	0.000026	0.000025	0.000024	0.000027	0.000026	0.000025	0.000009	0.000009	0.000008
phosphoglucomutase/phosphopentomutase	5.4.2.7	<i>PGM2</i>	0.000087	0.000086	0.000085	0.000187	0.000186	0.000185	0.000056	0.000056	0.000055
glucose-6-phosphate isomerase	5.3.1.9	<i>pgi</i>	0.000398	0.000395	0.000393	0.000446	0.000441	0.000438	0.000314	0.000312	0.000310
6-phosphofructokinase 1	2.7.1.11	<i>pfkA</i>	0.000197	0.000193	0.000192	0.000206	0.000202	0.000198	0.000185	0.000182	0.000180
fructose-bisphosphate aldolase, class II	4.1.2.13	<i>fbaA</i>	0.000386	0.000373	0.000368	0.000369	0.000359	0.000349	0.000301	0.000289	0.000282
triosephosphate isomerase (TIM)	5.3.1.1	<i>tpiA</i>	0.000338	0.000328	0.000322	0.000374	0.000365	0.000352	0.000273	0.000263	0.000259
methylglyoxal synthase	4.2.3.3	<i>mgsA</i>	0.000174	0.000161	0.000158	0.000196	0.000181	0.000176	0.000114	0.000107	0.000101
glyceraldehyde-3-phosphate dehydrogenase (NAD(P))	1.2.1.59	<i>gap2</i>	0.000002	0.000002	0.000003	0	0	0	0.000001	0.000001	0.000001
glyceraldehyde-3-phosphate dehydrogenase (NADP+)	1.2.1.9	<i>gapN</i>	0.000001	0.000001	0.000002	0.000002	0.000003	0.000004	0.000004	0.000004	0.000005
phosphoglycerate kinase	2.7.2.3	<i>pgk</i>	0.000341	0.000354	0.000363	0.000292	0.000299	0.000306	0.000328	0.000338	0.000346
2,3-bisphosphoglycerate-independent phosphoglycerate mutase	5.4.2.12	<i>gpmI</i>	0.000359	0.000373	0.000381	0.000330	0.000338	0.000343	0.000414	0.000423	0.000430
enolase	4.2.1.11	<i>eno</i>	0.000360	0.000375	0.000381	0.000346	0.000356	0.000365	0.000302	0.000309	0.000317
pyruvate kinase	2.7.1.40	<i>pyk</i>	0.000254	0.000268	0.000276	0.000465	0.000477	0.000488	0.000205	0.000218	0.000225
lactaldehyde dehydrogenase/glycolaldehyde dehydrogenase	1.2.1.22,1.2.1.21	<i>aldA</i>	0.000321	0.000346	0.000353	0.000232	0.000245	0.000252	0.000310	0.000333	0.000340
L-lactate dehydrogenase	1.1.1.27	<i>ldh</i>	0.000345	0.000361	0.000371	0.000535	0.000516	0.000544	0.000067	0.000075	0.000080

Note: EC is the description of KEGG enzyme number. C(M, I), C (M, I)G, and C(M, I)GM, represent anaerobic culture of intestinal digesta samples removed from the mice fed on the C(M, I) group diet using a medium supplied with 1.0% choline, a mixture of 1.0% glycine and 1.0% choline, another of 1.0% glycine, 1.5 mmol L⁻¹ methylglyoxal and 1.0% choline.

Table 8 The pearson correlation analysis among key enzymes expression of choline metabolism and enzymes associated with biosynthesis and utilization of glycine

	CutD	CutC	Methylglyoxal	Pyruvate	Glycine
CutD		.992**	.779*	.730	.754*
	1	.000	.013	.055	.019
CutC	.992**	1	.782*	.701	.688*
	.000		.016	.052	.040
Methylglyoxal	.779*	.782*	1	.772*	-.799*
	.013	.016		.015	.049
Pyruvate	.730	.701	.772*	1	.657
	.055	.052	.015		.173
Glycine	.754*	.688*	-.799*	.657	1
	.019	.040	.049	.173	
gbsB	.631	.539	.508	-.769	.907**
	.068	.134	.255	.115	.001
BHMT	.768*	.721*	-.692	-.329	.751*
	.016	.028	.154	.388	.020
PIPOX	.823**	.817**	-.484	-.615	.566
	.006	.007	.187	.078	.112
GNMT	.552	.532	-.795	-.596	.616
	.123	.140	.101	.206	.077
ItaE	-.411	-.296	-.536	-.640	-.814**
	.272	.439	.137	.181	.008
TDH	.448	.348	-.702*	-.561	.692
	.291	.358	.035	.116	.154
kbl	-.943**	-.958*	-.942**	-.636	-.831**
	.000	.021	.000	.184	.005
aofH	-.725*	-.731*	-.933*	.874	-.480
	.047	.020	.033	.062	.191
glyA	.630	.538	.512	-.738	.906**
	.069	.135	.253	.130	.001
SDS	-.485	-.382	-.421	-.441	.408
	.269	.311	.259	.295	.276
sdaA	.729*	.686*	.943**	.882*	.957**
	.026	.042	.000	.015	.000
crr	.713*	.645	.985**	.677	.960**
	.031	.061	.000	-.162	.000
ptsGb	.744	.986	.713*	.525	.698*
	.127	.007	.031	.245	.036
PGM2	.449	.513	.973**	-.575	.960**
	.225	.158	.000	.217	.000
pgi	.424	.412	.958**	.851	.866**
	.255	.313	.000	.074	.003
pfkA	.407	.416	.948**	-.751	.799**

	.277	.311	.000	.124	.010
fbaA	-.755	-.539	.788*	.458	.491
	.122	.237	.012	.284	.180
tpiA	.386	.466	.950**	-.993	.828**
	.304	.280	.000	.368	.006
mgsA	.447	.639	.922**	.911	.766*
	.292	.182	.000	.435	.016
gapN	.489	.575	-.758*	-.442	-.751
	.182	.106	.018	.294	.124
gap2	-.901**	-.926**	.635	.675*	-.601
	.001	.000	.066	.046	.087
pgk	-.870**	-.859**	-.743*	.684*	-.734*
	.002	.003	.022	.042	.024
gpmI	-.430	-.328	-.963**	.868	-.842**
	.248	.389	.000	.065	.004
eno	-.799	.560	.921**	.625	.514
	.099	-.225	.000	.072	.157
pyk	.817**	.741*	.954**	.970**	-.635
	.007	.022	.000	.000	.184
aldA	-.860**	-.828**	-.845**	-.811**	.573
	.003	.006	.004	.008	.107
ldh	.458	.345	.960**	.884**	.776
	.215	.363	.000	.002	.111

** . Correlation is significant at the 0.01 level (2-tailed). * . Correlation is significant at the 0.05 level (2-tailed).