

Table S1. Major differential metabolites in serum

Metabolite	FC ¹	<i>p</i> ²	VIP ³	Up/Down
CON-HG				
Salicylic acid	2.44	<0.01	1.61	up
Catechol	1.79	0.01	1.30	up
4-Methylcatechol	2.06	0.03	1.49	up
4-Methylphenol	1.82	<0.01	1.63	up
o-Cresol	1.55	0.01	1.60	up
HG.vs.HGE				
Guanine	0.40	0.03	1.78	down
Hypoxanthine	0.42	0.04	1.71	down
Guanosine	0.04	<0.01	2.78	down
Inosine	0.53	0.04	1.66	down
3'-AMP	0.64	0.06	1.15	down

¹FC = fold change is the ratio of the mean values of all biological repeated quantitative values of each metabolite in the test period.

²*p*-value represents the significance level of difference between the two groups

³VIP refers to the variable projection importance of the first principal component of the PLS-DA model

Table S2. Common differential metabolites of CON-HG and HG-HGE

Metabolite	CON-HG			HG-HGE		
	FC ¹	<i>p</i> ²	VIP ³	FC	<i>p</i>	VIP
N-Phenylacetylglutamine	2.92	<0.01	1.82	0.66	0.03	1.08
1-(4-benzylpiperazino)-2-(pyridin-2-ylamino)propan-1-one	0.61	<0.01	1.30	0.62	<0.01	1.45
4-Methylcatechol	2.06	0.03	1.49	0.46	0.02	2.06
Choline	1.79	0.03	1.49	0.58	0.02	1.89
3-Methyladipic acid	0.37	<0.01	1.87	2.05	0.01	2.02
4-Methylphenol	1.82	<0.01	1.63	0.50	<0.01	2.49
2-Mercaptobenzothiazole	0.04	<0.01	2.28	2.46	0.04	1.03
P-Coumaroyl Agmatine	2.17	0.01	1.26	1.51	0.02	1.12
N-{4-[(2R,3R)-3-(Hydroxymethyl)-5-oxo-2-morpholinyl]phenyl}acetamide	2.88	0.02	1.60	0.51	<0.01	1.63
DL-o-Tyrosine	0.20	<0.01	1.91	3.18	0.02	1.75
2-[(3-methylbenzo[b]thiophen-2-yl)carbonyl]benzoic acid	2.76	0.02	1.47	0.33	0.04	1.77
Oxadipic Acid	0.30	<0.01	1.82	2.83	0.01	2.13
Ureidoisobutyric Acid	0.15	<0.01	2.08	2.95	0.01	1.53
Stearic acid	2.03	<0.01	1.56	0.51	0.05	1.64
D-Phenylalanine	0.16	<0.01	2.08	3.02	0.02	1.49
Cystine	0.41	<0.01	2.03	2.17	<0.01	2.18
11-Epiprostaglandin E1	1.60	0.04	1.25	0.57	0.04	1.87
trans-3-Hydroxycotinine	0.27	<0.01	2.38	1.50	0.01	1.05
L-Tryptophan	0.23	0.01	1.70	3.42	0.02	1.92
11(Z),14(Z),17(Z)-Eicosatrienoic acid	0.50	<0.01	1.71	2.11	<0.01	2.45
LPC 10:0	0.52	0.02	1.03	3.10	<0.01	2.51
octadec-9-ynoic acid	0.64	0.01	1.12	2.36	<0.01	2.95
(+)-alpha-Lipoic acid	0.45	0.01	1.57	1.89	0.03	1.74
Lysopa 18:0	1.81	<0.01	1.22	0.35	<0.01	2.56
LPC 13:0	0.50	0.03	1.23	1.93	0.03	1.66
Ethyl 3-methyl-5-[(phenoxycarbonyl)amino]isothiazole-4-carboxylate	1.53	0.03	1.43	0.56	0.01	2.33
3,8,9-trihydroxy-10-propyl-3,4,5,8,9,10-hexahydro-2H-oxecin-2-one	0.44	0.02	1.38	2.26	0.02	2.00
NNK	2.21	<0.01	1.11	1.98	<0.01	1.54
L-Glutathione (reduced)	1.74	<0.01	1.77	0.63	<0.01	2.12

¹FC is the ratio of the mean values of all biological repeated quantitative values of each metabolite in the test period.

²*p*-value represents the significance level of difference between the two groups

³VIP refers to the variable projection importance of the first principal component of the PLS-DA model

Table S3. Effects of vitamin E on rumen microflora phylum levels induced by high concentration in subacute ruminal acidosis cows

Phylum	Dietary treatments ¹			SEM ²	p-Value	
	CON	HG	HGE		CON vs. HG	HG vs. HGE
<i>Bacteroidota</i>	48.18	48.13	45.68	0.75	0.98	0.20
<i>Firmicutes</i>	43.21	35.98	32.70	1.64	0.04	0.30
<i>Proteobacteria</i>	1.12	5.10	13.44	1.56	0.06	<0.01
<i>Spirochaetota</i>	1.66	2.14	1.02	0.22	0.34	0.04
<i>Actinobacteriota</i>	1.03	2.72	1.83	0.29	0.01	0.15
<i>Fibrobacterota</i>	0.88	1.29	0.46	0.17	0.29	0.04
<i>Cyanobacteria</i>	0.20	0.61	0.46	0.12	0.18	0.62
<i>Euryarchaeota</i>	1.04	0.77	0.59	0.09	0.18	0.37
<i>unidentified_Bacteria</i>	0.91	0.91	0.92	0.02	0.96	0.96
<i>Desulfobacterota</i>	0.11	0.09	0.11	0.01	0.44	0.58

¹ CON = Control stage, HG = High-grain diet, HGE = High vitamin E diet.

² SEM standard error of the mean.

Table S4. Effects of vitamin E on genus level of rumen microflora induced by high concentration in subacute ruminal acidosis cows

Genus	Dietary treatments ¹			SEM ²	p-Value	
	CON	HG	HGE		CON vs. HG	HG vs. HGE
<i>Prevotella</i>	19.45	25.12	24.55	1.01	0.01	0.78
<i>Succinivibrionaceae_UCG-001</i>	0.33	3.94	10.55	1.23	0.01	<0.01
<i>Rikenellaceae_RC9_gut_group</i>	7.69	4.20	3.63	0.54	<0.01	0.43
<i>Treponema</i>	1.61	2.11	0.98	0.22	0.32	0.04
<i>Succiniclasicum</i>	3.62	2.50	5.30	0.49	0.29	0.02
<i>Ruminococcus</i>	7.39	1.29	3.88	0.47	<0.01	0.38
<i>Fibrobacter</i>	0.88	0.61	0.46	0.17	0.30	0.04
<i>Olsenella</i>	0.41	1.28	0.35	0.16	0.01	0.01
<i>Pseudoscardovia</i>	0.08	0.92	0.86	0.16	0.03	0.87
<i>unidentified_Chloroplast</i>	0.12	0.57	0.36	0.12	0.15	0.48
<i>Succinivibrionaceae_UCG-002</i>	0.41	0.51	1.08	0.12	0.71	0.06
<i>Christensenellaceae_R-7_group</i>	3.92	1.93	1.29	0.32	<0.01	0.06
<i>Acetitomaculum</i>	3.46	2.61	1.93	0.20	0.02	0.04
<i>Prevotellaceae_UCG-001</i>	2.10	1.88	1.93	0.11	0.44	0.88
<i>unidentified_Mitochondria</i>	0.00	0.02	0.42	0.09	0.93	0.07
<i>NK4A214_group</i>	2.79	1.51	1.09	0.21	<0.01	0.07
<i>Selenomonas</i>	0.57	1.34	0.73	0.10	<0.01	<0.01
<i>Lachnospiraceae_NK3A20_group</i>	1.92	1.88	1.09	0.12	0.82	<0.01
<i>Saccharofermentans</i>	2.17	1.47	1.18	0.14	0.01	0.18
<i>Prevotellaceae_NK3B31_group</i>	1.26	1.31	0.87	0.06	0.52	<0.01
<i>CAG-352</i>	0.41	0.19	0.39	0.04	0.02	0.03
<i>Methanobrevibacter</i>	0.90	0.70	0.55	0.07	0.25	0.42
<i>Bifidobacterium</i>	0.28	0.35	0.37	0.03	0.47	0.82
<i>Dialister</i>	0.10	0.56	0.21	0.06	<0.01	<0.01
<i>Shuttleworthia</i>	0.37	0.73	0.79	0.07	0.01	0.63
<i>Ruminobacter</i>	0.21	0.34	0.20	0.03	0.09	0.06
<i>Enterobacter</i>	0.00	0.00	0.08	0.03	0.98	0.25
<i>Erysipelotrichaceae_UCG-007</i>	0.03	0.14	0.29	0.04	0.15	0.04
<i>Butyrivibrio</i>	1.10	0.81	0.49	0.07	<0.01	<0.01
<i>UCG-005</i>	1.14	0.59	0.49	0.09	<0.01	0.33
<i>Others</i>	35.27	34.77	33.64	0.34	0.52	0.15

¹ CON = Control stage, HG = High-grain diet, HGE = High vitamin E diet.

² SEM standard error of the mean.