Electronic Supplementary Material (ESI) for Food & Function.

Supplementary Table 1 Composition and nutrients contents of the basal diet

Ingredients	Basal diet	Analyzed chemical composition	
Corn	61.00	Dry matter	85.99
Soybean meal	10.00	Crude protein[2]	18.89
Extruded soybean	8.00	Crude fiber	2.20
Fermented soybean	5.00	Net energy	2330.03
Whey powder	8.00	Digestive energy (kcal/kg）	3467.31
Fish meal	3.00		
Soybean oil	0.70		
Sucrose	1.00		
Premix[1]	1.00		
Limestone power	0.51		
Total	100		

[1] Premix provided the following amounts of vitamins and trace minerals per kg of the complete diet: Organic trace minerals 0.15%; Piglets vitamin 0.04%; Carrier 0.38%; Antioxidant 0.1%; Calcium dihydrogen phosphate 0.78%; Salt 0.37%; Lysine HCL（98%） 0.64%.

[2] The analyzed composition of amino acids (%, as-fed basis) in the basal diet was as follows: alanine, 0.86; arginine, 1.35; aspartate plus asparagine, 1.87; cysteine, 0.18; glutamate plus glutamine, 3.01; glycine, 0.91; histidine, 0.43; isoleucine, 0.80; leucine,1.47; lysine, 2.02; methionine, 0.21; phenylalanine, 0.87; proline, 0.89; serine, 0.92; threonine, 0.74; tyrosine, 0.47; and valine, 0.79.

Supplementary Table 2 Primers of pig used in this study

Gene Name	Accession No.	Sequence (5'-3')	Size
GAT1	XM_005669778.2	CTGGACTGGAAAGGTGGTCT GCGGAAGTTGGGTGTGATA	130
GAT2	XM_003126576.4	CAGTTTGTGTGCGTGGAAAG ACGAGGAAGGAGGTGACAGA	119
GAT3	XM_005657080.1	GGGCATTCCTGATTCCCTAT GGCACACTTTCCTCCAACAC	123
BGT1	XM_003126574.4	GATGGAGTTCGTGCTGTCTG CCGCAGGTGAAGAAGAAGAT	132
pIgR	NM_214159.1	AACCTCACCAACTTCCCAGAG CTAATGCCCAGACCACACTTG	98
MUC4	XM_013989745.1	GGCTGCTCATTGAGAGGAGT ATGTTCCCGAACTCCAAGG	214
MUC2	NM_001206344.1	AGGATGCCCAATGGCTCTACT AAGGAGGCTGGTTCCGTTGAT	96
MUC1	XM_001926883.5	ACACCCATGGGCGCTATGT GCCTGCAGAAACCTGCTCAT	68
IL-1	NM_001302388.1	AAAGGGGACTTGAAGAGAG CTGCTTGAGAGGTGCTGATGT	286
IL-18	NM_213997.1	TATGCCTGATTCTGACTGTT ATGAAGACTCAAACTGTATCT	260
TNF-α	NM_214022.1	TTCCTCACTCACACCATCAGCC TGCCCAGATTCAGCAAAGTCC	224
IL-2	NM_213861.1	CTGGATTTACAGTTGCTTTTG AGTCAGTGTTGAGTAGATGCTTT	348
IFN-γ	NM_213948.1	GGCCATTCAAAGGAGCATGG GATGGCTTTGCGCTGGATCT	139
IL-10	NM_214041.1	CATCCACTTCCCAACCAGCC CTCCCCATCACTCTCTGCCTTC	220
IL-6	NM_001252429.1	TGGCTACTGCCTTCCCTACC CAGAGATTTTGCCGAGGATG	132
IL-4	NM_214123.1	TCCACGGACACAAGTGCGAC TGTTTGCCATGCTGCTCAGG	243
IL-23	NM_001130236.1	ACAGCAGCTCTGCACGCTGG CACAGCCATCCCCGCACTGG	125
IL-17	NM_001005729.1	ATCCTCGTCCCTGTCACTGC ACATGCTGAGGGAAGTTCTTGTC	120
IL-22	XM_001926156.1	GATGAGAGAGCGCTGCTACCTGG GAAGGACGCCACCTCCTGCATGT	112
TLR2	NM_213761.1	TGCTATGACGCTTTCGTGTC CGATGGAGTCGATGATGTTG	163
TLR4	XM_013986843.1	TCAGTTCTCACCTTCCTCCTG GTTCATTCCTCACCCAGTCTTC	166
TLR5	NM_001123202.1	CCTTCCTGCTTCTTTGATGG CTGTGACCGTCCTGATGTAG	124

TLR6	NM_213760.1	AACCTACTGTCATAAGCCTTCATTC	Tlr6
		GTCTACCACAAATTCACTTTCTTCAG	
MyD88	XM_013992286.1	GATGGTAGCGGTTGTCTCTGAT	148
		GATGCTGGGGAACTCTTTCTTC	
ACTB	XM_003124280.4	CTGCGGCATCCACGAAACT	147
		AGGGCCGTGATCTCCTTCTG	
GAPDH	NM_001206359.1	ATCCTGGGCTACACTGAGGAC	104
		AAGTGGTCGTTGAGGGCAATG	

Primers were designed under the principle of primer design with Primer 6.0 software (PRIMER-E, New Zealand) and Oligo 5.0 software (Molecular Biology Insights, Inc., USA) according to the gene sequences of sus scrofa on GenBank.

Supplementary Table 3. Effects of GABA on serum amino acid profile in ETEC-challenged piglets（μg/ml）

	E	**GE**	**t Value**	**P-value**
L-Histidine	7.66±0.74	6.26±0.81	1.28	0.2223
L-Serine	18.5±2.38	18.24±2.99	0.07	0.9456
L-Arginine	23.18±2.03	25.82±3.25	-0.69	0.5033
Glycine	34.14±2.92	39.47±3.04	-1.26	0.2342
L-Aspartic Acid	5.1±0.67	5.58±0.75	-0.48	0.6364
Glu	41.66±3.31	46.91±6.72	-0.7	0.4973
L-Alanine	38.23±3.78	33.01±3.92	0.96	0.3565
L-Proline	23.44±2.05	23.45±3.28	0	0.998
L-Cystine	1.69±0.44	1.76±0.32	-0.13	0.9016
L-Lysine	37.72±4.24	33.32±5.27	0.65	0.5273
L-Tyrosine	18.31±1.62	20.33±3.19	-0.59	0.5661
L-Methionine	7.72±0.61	8.13±1.2	-0.31	0.7615
L-Valine	19.55±1.72	22.7±2.9	-0.93	0.3687
L-isoleucine	11.61±0.54	13.63±1.71	-1.13	0.2962
L-leucine	18.63±1.07	18.71±2.62	-0.03	0.9767
L-phenylalanine	14.75±1.87	13.53±1.7	0.48	0.6408
L-Tryptophan	6.49±1.03	6.33±1.03	0.11	0.9152

Amino acid profile of the serum was detected by HPLC. Piglets from E group (n=10) and GE group (n=8) accessed to a basal diet supplemented without and with GABA, separately. Unpaired t-test was used for analysis of the date (Mean ± SEM). Dietary GABA supplementation reduced the concentration of threonine, while did not affect other serum amino acids profiles.

Supplementary Table 4. Gene expression of the GABA transporters in the small intestine of ETEC-challenged piglets.

	Con	**GABA**	**t Value**	**P value**
Jejunum				
GAT1	1±0.1	1.33±0.18	-1.68	0.1177
GAT3	1±0.15	1.05±0.19	-0.19	0.8533
BGT1	1±0.13	1.07±0.23	-0.29	0.7786
Ileum				
GAT1	1±0.09	0.92±0.06	0.74	0.4706
GAT2	1±0.09	1.24±0.16	-1.39	0.195
GAT3	1±0.29	0.64±0.17	0.99	0.3405
BGT1	1±0.08	1.29±0.26	-1.2	0.2514

RT-PCR analyzed related gene expressions of GATs. Piglets from E group (n=10) and GE group (n=8) accessed to a basal diet supplemented without and with GABA, separately. Unpaired t-test was used for analysis of the data (Mean ± SEM).

Supplementary Table 5 Relative expression of the immunity-related gene in the small intestine of ETEC-challenged piglets.

	Con	GABA	t Value	P-value
Jejunum				
IL-1	1±0.11	0.74±0.18	1.31	0.2134
IL-2	1±0.1	1.06±0.15	-0.33	0.7456
IL-6	1±0.09	1.08±0.16	-0.46	0.6563
IL-10	1±0.09	0.81±0.08	1.47	0.1658
IL-17	1±0.19	1.15±0.32	-0.41	0.6861
IL-18	1±0.21	1.24±0.54	-0.42	0.6884
IL-22	1±0.13	0.71±0.15	1.09	0.959
TNF-α	1±0.1	0.78±0.09	1.59	0.1349
IFN-γ	1±0.11	0.83±0.1	1.13	0.2795
pIgR	1±0.08	1.15±0.21	-0.67	0.5202
MUC1	1±0.1	1.53±0.28	-1.75	0.1204
MUC2	1±0.07	1.37±0.24	-1.47	0.1839
MUC4	1±0.33	2.68±1.12	-1.44	0.1941
TLR2	1±0.09	1.12±0.07	-1.01	0.3316
TLR4	1±0.05	1.13±0.13	-0.96	0.3713
TLR5	1±0.05	0.95±0.09	0.47	0.6428
TLR6	1±0.07	1.14±0.12	-1.03	0.323
MyD88	1±0.01	1.1±0.1	-1.05	0.3331
Ileum				
IL-1	1±0.2	0.6±0.04	1.93	0.0923
IL-2	1±0.08	1.15±0.32	-0.47	0.6561
IL-4	1±0.1	1.53±0.43	-1.21	0.2661
IL-6	1±0.03	1.17±0.17	-1.01	0.3581
IL-10	1±0.05	1.05±0.06	-0.6	0.5628
IL-17	1±0.18	0.96±0.28	0.12	0.9099
IL-22	1±0.22	1.13±0.18	-0.45	0.6614
MUC1	1±0.17	1.34±0.13	-1.56	0.1404
MUC4	1±0.28	1.75±0.83	-0.86	0.4178
TLR2	1±0.09	0.96±0.15	0.21	0.8335
TLR4	1±0.19	1.29±0.18	-1.12	0.281
TLR5	1±0.1	1.02±0.15	-0.09	0.9265
TLR6	1±0.07	0.83±0.1	1.33	0.205
MyD88	1±0.08	1.2±0.13	-1.37	0.1923

Related gene expressions of interleukins, mucin, TLRs, and MyD88 in the jejunum and ileal of piglets were analyzed by RT-PCR. Piglets from E group (n=10) and GE group (n=8) accessed to a basal diet supplemented without and with GABA, separately. Unpaired t-test was used for analysis of the data (Mean ± SEM).

Supplementary Table 6 Data pre-processing statistics and quality control

Sample Name	Raw PE(#)	Raw Tags(#)	Clean Tags(#)	Effective Tags(#)	Base(nt)	AvgLen (nt)	Q20	Q30	GC %	Effective %
E1	79,205	71,446	62,275	58,502	24,371,636	417	98.38	96.78	52.5	73.86
E2	85,268	77,356	69,548	67,084	27,517,098	410	98.49	97.02	52.6	78.67
E3	72,516	64,889	57,917	56,926	23,428,232	412	98.42	96.90	52.0	78.50
E4	75,354	68,783	61,424	60,572	24,900,776	411	98.42	96.88	52.7	80.38
E5	86,181	77,474	70,074	69,365	28,225,213	407	98.41	96.88	53.0	80.49
E6	72,529	65,163	59,555	59,061	23,901,351	405	98.41	96.91	53.1	81.43
E7	70,549	63,340	57,808	57,106	23,210,336	406	98.45	96.98	53.0	80.95
E8	76,277	68,352	62,125	61,474	24,975,630	406	98.41	96.91	53.1	80.59
GE1	74,291	67,261	58,576	57,311	23,876,996	417	98.39	96.84	52.6	77.14
GE2	74,454	67,088	58,793	56,101	23,290,905	415	98.38	96.82	52.8	75.35
GE3	70,933	64,250	57,008	56,525	23,306,702	412	98.40	96.84	52.9	79.69
GE4	70,701	63,294	56,895	55,971	22,918,099	409	98.41	96.90	52.6	79.17
GE5	87,664	79,800	72,675	72,179	29,308,984	406	98.44	96.94	53.1	82.34
GE6	80,553	72,892	63,440	60,760	25,279,814	416	98.34	96.72	52.8	75.43

Raw PE, Raw Tags, Clean Tags, Effective Tags, Base(nt), AvgLen (nt), Q20, Q30 , GC%, and Effective% of each sample in E group (n=8) and GE group (n=6).

Supplementary Table 7 Microbial species distribution in ileal content of ETEC-challenged piglets

	E	**GE**	**Z score**	**P value**
Phylum				
Firmicutes	0.9371	0.7079	-2.9047	0.0037
Cyanobacteria	0.0049	0.1031	2.001	0.0454
Proteobacteria	0.0429	0.0984	1.8719	0.0612
Actinobacteria	0.0013	0.0231	3.0338	0.0024
Tenericutes	0.0097	0.0309	1.0973	0.2725
Euryarchaeota	0.0001	0.0116	2.3393	0.0193
Bacteroidetes	0.0018	0.0114	2.6465	0.0081
Fusobacteria	0.0011	0.0035	2.6465	0.0081
Spirochaetes	0.0001	0.0027	0.3877	0.6982
Saccharibacteria	0.0003	0.0022	1.1047	0.2693
Others	0.0005	0.0052	--	--
Class				
Clostridia	0.7674	0.4883	-2.001	0.0454
Erysipelotrichia	0.0902	0.0541	-0.3227	0.7469
Chloroplast	0.0049	0.103	2.001	0.0454
Bacilli	0.0749	0.1452	0.9682	0.3329
Gammaproteobacteria	0.0388	0.0805	1.2264	0.22
unidentified_Actinobacteria	0.001	0.0191	2.1348	0.0328
Mollicutes	0.0097	0.0309	1.0973	0.2725
Methanobacteria	0.0001	0.0115	2.4177	0.0156
Negativicutes	0.0046	0.0195	2.3883	0.0169
Bacteroidia	0.0015	0.0095	2.1301	0.0332
Others	0.0068	0.0384	--	--
Genus				
Clostridium_sensu_stricto_1	0.6525	0.2714	-2.6465	0.0081
Turicibacter	0.0889	0.048	-0.71	0.4777
Romboutsia	0.052	0.0958	-0.0645	0.9485
unidentified_Chloroplast	0.0049	0.1029	2.0032	0.0452
Terrisporobacter	0.0512	0.0836	0	1
Lactobacillus	0.0594	0.0998	0.4518	0.6514
Escherichia-Shigella	0.0173	0.0321	0	1
Mycoplasma	0.0097	0.0306	1.0973	0.2725
Streptococcus	0.0095	0.0248	2.001	0.0454
Rothia	0.0003	0.0117	2.2642	0.0236
Methanobrevibacter	0.0001	0.0115	2.4177	0.0156
Veillonella	0.0044	0.0172	1.7428	0.0814
Pediococcus	0.0028	0.0064	1.682	0.0926
Shewanella	0.0014	0.008	0	1
Actinobacillus	0.0085	0.0097	0.4518	0.6588
Others	0.0372	0.1465	--	--

Top 10 microbial population in E group (n=8) and GE group (n=6) on the phylum level and class level, and top 15 microbial population of E group (N=8) and GE group (n=6) on genus level. The Wilcoxon test was used for analysis of the data (Mean ± SEM). *: $P<0.05$.