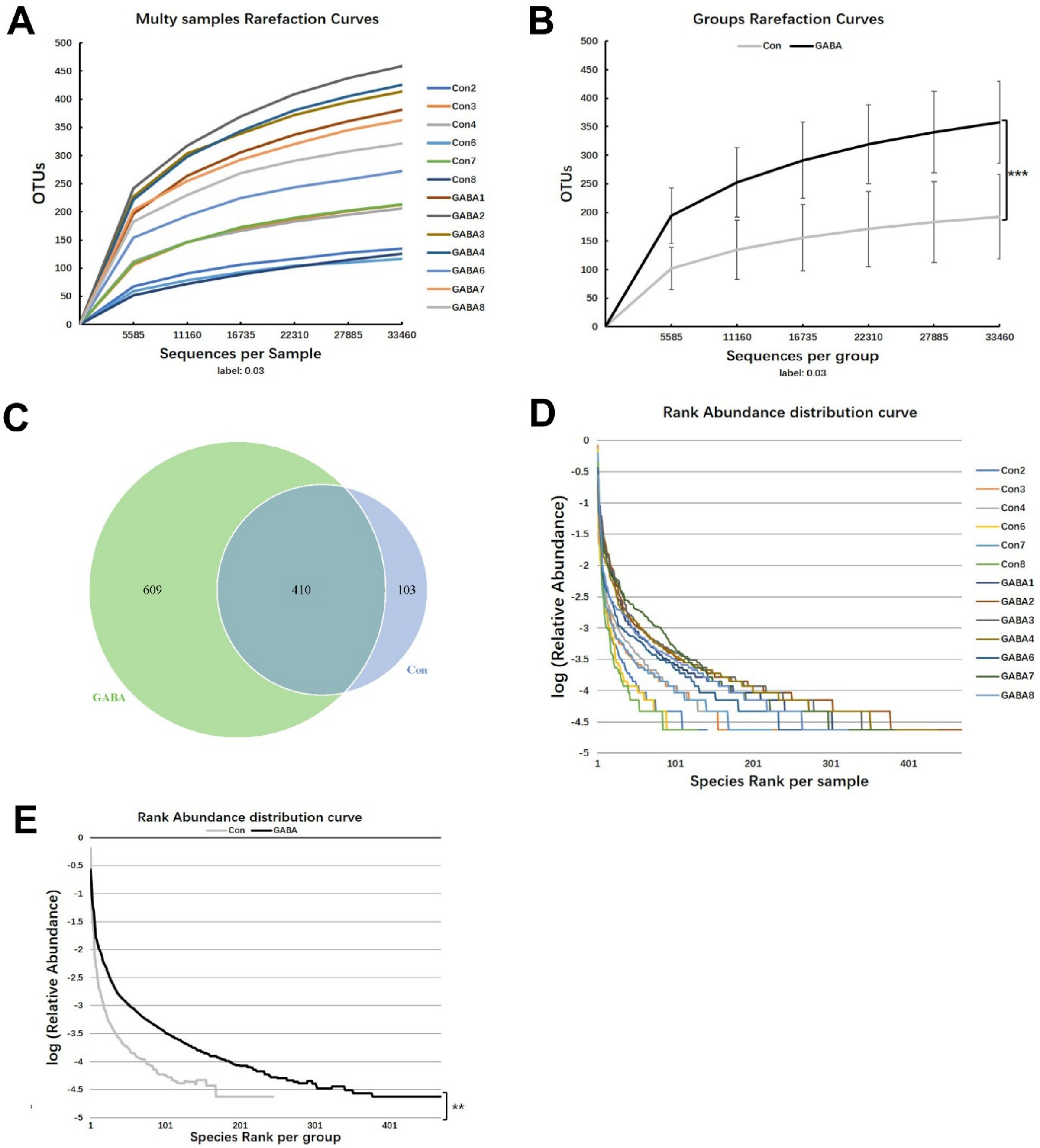
A
Multy samples Rarefaction Curves
OTUs
0
50
100
150
200
250
300
350
400
450
500
5585
11160
16735
22310
27885
33460
Sequences per Sample
label: 0.03
Con2
Con3
Con4
Con6
Con7
Con8
GABA1
GABA2
GABA3
GABA4
GABA6
GABA7
GABA8
B
Groups Rarefaction Curves
Con
GABA
OTUs
Sequences per group
label: 0.03
***
C
609
410
103
GABA
Con
D
Rank Abundance distribution curve
log (Relative Abundance)
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
1
101
201
301
401
Species Rank per sample
E
Rank Abundance distribution curve
Con
GABA
log (Relative Abundance)
Species Rank per group
**

Figure S1 Dietary GABA supplementation highly regulates composition and alpha diversity of the intestinal microbiota. Rarefraction curve (A, per sample; B, group) revealed that sequencing data, sequencing depth and coverage met the demand of the followed analysis. Venn graph (C) illustrated the general profiles of OTUs between Con group and GABA group. Rank abundance curve (C, per sample; D, group) shown that GABA altered Alpha diversity of gut microbome. Con, control group (N=6); GABA, GABA group (N=7). **: P<0.01;***: P<0.001.

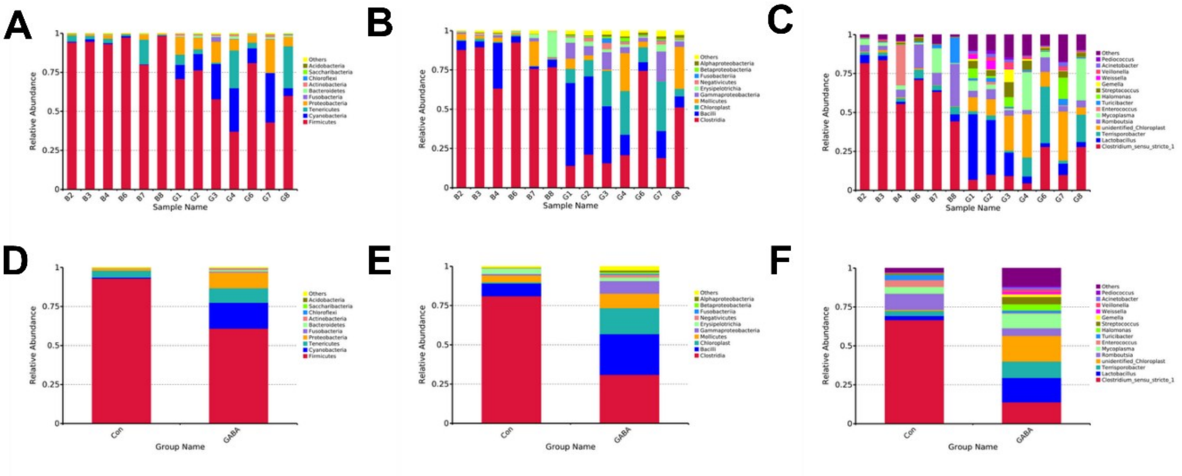

Figure S2 Relative contribution of intestinal microbiota in content of colon in control group (N=6) and GABA group (N=7). Relative contribution of microbiota in each sample (phylum, A; order, B; genus, C) and in each group (phylum, D; order, E; genus, F); Con, control group (N=6); GABA, GABA group (N=7).

Table S1 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 (kcal/kg)	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	100.00		

[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.

Table S2 Primers used in this study

Gene Name	Accession No.	Sequence (5'-3')	Size (bp)
GAD1	NM_213894.1	GAGCGAACAGGTTGGAGAAG AGAAGAGCAGGCTTGTGAGC	126
GAD2	NM_213895.2	AGACAAGGCCTTACAGTGCG TGGCCTTAATCACTGGAGCC	195
SLC6A12	XM_003126574.4	GATGGAGTTCGTGCTGTCTG CCGCAGGTGAAGAAGAAGAT	132
SLC6A11	XM_005657080.1	GGGCATTCCTGATTCCCTAT GGCACACTTTCCTCCAACAC	123
SLC6A13	XM_003126576.4	CAGTTTGTGTGCGTGGAAAG ACGAGGAAGGAGGTGACAGA	119
SLC6A1	XM_005669778.2	CTGGACTGGAAAGGTGGTCT GCGGAAGTTGGGTGTGATA	130
GABAB1	NM_001123114.1	GCCCGTGGACTATGAGATTG CTTGGAGCAGATTCGGACAC	136
GABAB2	XM_003122032	CATCACCCTTTGCCTGGTGT AGGTGGTCTTTTCTGGTGTGT	275
GABAAGT	XM_013984876	GCCTTCCCATTGCTGTTTGG GCAGAGGACGGAACAACAGA	132
GABAAGG	XM_013978644	GGATGATGGGACTCTGCTGT TCAGAGGACACGAGTGAGCA	101
GABAAGG	XM_003135469	TGGCCAAGGGGAATCAAGAC TCGGTCACTGCATCTCCAAG	195
GABAACA	XM_003356906	ACCTCAGACGGAAGATGGGT GCTCAGTGTGGTCATGGTGA	158
GABAAGT	XM_003121648	GGCTAAACCGAGAATCAGTCC ACACGGCTATGAACCAGTCC	141
GABAAGC	XM_003134090	GGCATGCTTGTCCACTCAAG GGCCGGCACAGATTCCTTAT	314
GABAAGC	XM_013984877	GCTGCTAGTGCCAACAATGA GTTGGGGAGAGGTTTCCAGT	122
GABAAG	XM_013988717	TCTCCTACAACCACACCAACG AGCCTGATGAGCTTGTTCTCC	139
GABAAG	XM_013986487	ACCTGGTGAGCCTGTTATGG GTACAACACCTTGCCGTCCT	122
GABAAAC	XM_003128952.5	CAAAACTTGGGTCTTGGCACC TACTGTGGGCCTCACTCCTA	118
GABAATG	XM_003359825	CTCCCAATAGGATGCTGAGG GGCAGGAGTGTTCATCCATT	120
GABAAGA	XM_005659794	ATGGGCCTCAGAAACACCAC CACGAAGAGGTCCATAGCGG	291
GABAAA	XM_003134060.4	CTTCACTTTGGATGCACGCC ACAGGACTGTGCCATTGGAG	137
GABAAA	XM_013986506	AGAGGCAACTCACAAGAGCC GGGGGACTTTCCTCATCAGC	151
GABAAAC	XM_013992689.1	AACGACCTGGGCAACTACAC TGCTCCTCTGGCTCTTCCT	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 GTCTACCACAAATTCACTTTCTTCAG	95
MyD88	XM_013992286.1	GATGGTAGCGGTTGTCTCTGAT GATGCTGGGGAACTCTTTCTTC	148
pIgR	NM_214159.1	AACCTCACCAACTTCCCAGAG CTAATGCCCAGACCACACTTG	98
Muc 4	XM_013989745.1	GGCTGCTCATTGAGAGGAGT ATGTTCCCGAACTCCAAGG	214
Muc 2	NM_001206344.1	AGGATGCCCAATGGCTCTACT AAGGAGGCTGGTTCCGTTGAT	96
Muc 1	XM_001926883.5	ACACCCATGGGCGCTATGT GCCTGCAGAAACCTGCTCAT	68
IL-1	NM_001302388.1	AAAGGGGACTTGAAGAGAG CTGCTTGAGAGGTGCTGATGT	286
IL-18	NM_213997.1	TATGCCTGATTCTGACTGTT ATGAAGACTCAAACTGTATCT	260
TNF-A	NM_214022.1	TTCCTCACTCACACCATCAGCC TGCCCAGATTCAGCAAAGTCC	224
IL-2	NM_213861.1	CTGGATTTACAGTTGCTTTTG AGTCAGTGTTGAGTAGATGCTTT	348
IFN-A	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
ACTB	XM_003124280.4	CTGCGGCATCCACGAAACT AGGGCCGTGATCTCCTTCTG	147
GAPDH	NM_001206359.1	ATCCTGGGCTACACTGAGGAC AAGTGGTCGTTGAGGGCAATG	104

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.

Table S3 Paired-end reads assembly and quality control

Sample Name	Raw PE(#)	Raw Tags(#)	Clean Tags(#)	Effective Tags(#)	Base(nt)	AL (nt)	Q20	Q30	GC%	Effective %
Control group										
Con2	76,850	69,682	63,673	63,197	25,691,450	407	98.42	96.90	52.70	82.23
Con3	89,171	81,210	74,470	73,676	29,893,179	406	98.47	97.02	52.92	82.62
Con4	75,639	68,830	61,130	60,379	24,900,382	412	98.41	96.87	52.71	79.83
Con6	80,291	73,490	67,785	67,289	27,258,863	405	98.53	97.11	53.05	83.81
Con7	56,619	51,354	46,495	45,818	18,736,673	409	98.43	96.95	51.66	80.92
Con8	78,612	71,536	64,566	64,038	26,197,248	409	98.42	96.90	52.87	81.46
GABA group										
GABA1	85,203	77,446	66,776	63,694	26,909,747	422	98.38	96.75	51.44	74.76
GABA2	76,566	64,542	55,804	53,008	22,281,080	420	98.35	96.73	51.38	69.23
GABA3	78,750	70,843	61,059	59,038	24,685,709	418	98.25	96.54	52.70	74.97
GABA4	79,466	71,534	63,259	61,803	25,717,880	416	98.48	97.05	51.32	77.77
GABA6	52,976	47,745	43,343	42,672	17,358,252	407	98.42	96.92	52.83	80.55
GABA7	82,775	74,201	64,816	62,697	26,061,677	416	98.35	96.74	53.88	75.74
GABA8	63,949	57,177	50,880	49,775	20,586,971	414	98.43	96.96	50.80	77.84
Average	75,144	67,661	60,312	59,006	24,329,162	412				
SUM	976,867	879,590	784,056	767,084	316,279,111					

Paired-end reads from illumina high-throughput sequencing were processed by data split, sequence assembly, data filtration and chimera removal to obtain effective tags. Piglets from control group (Con2-4, 6-8) and GABA group (GABA1-4,6-8) accessed to basal diet supplemented without and with GABA, separately. AL, Average length

Table S4 Pyrosequencing data and diversity indices of the microbiota in ileal content of piglets

Indices	**Con**	**GABA**	**SEM**	**P (T-test)**	**P (Wilcoxon)**
Observed species	179.67	387.57	58.43	0.000	0.001
Goods coverage	0.999	0.998	0.00	0.023	0.045
Chao1	231.69	434.87	85.11	0.002	0.005
AEC	231.05	447.83	69.90	0.000	0.002
Shannon	1.85	4.19	0.63	0.000	0.001
Simpson	0.50	0.86	0.15	0.002	0.001
PD whole tree	22.38	44.63	11.27	0.002	0.002

Richness indices (Observed species, Goods coverage, Chao1 and AEC) and diversity indices (Shannon, Simpson and PD whole tree) were statistically calculated by mothur. Piglets from control group (Con, n=6) and GABA group (GABA, n=7) accessed to basal diet supplemented without and with GABA, separately. Both unpaired t-test and Wilcoxon nonparametric test were used for analyzation of the data. *: $P<0.05$. AEC, Abundance-based Coverage Estimator; PD whole tree, phylogenetic diversity whole tree.