Tables

Table 1 Composition and nutrient levels of the experimental basal diet (as-fed basis unless stated otherwise, %).

Item			
Ingredients **(**%)		**Calculated Nutrient levels[3]**	
Corn (CP 8.0%)	61.00	Metabolizable energy (kcal/kg)	2.9072
Soybean meal (CP 53.0%)	33.00	Crude protein (%)	19.24
Soybean oil	2.00	Total Calcium (%)	0.97
Limestone-calcium carbonate	1.21	Available phosphorus (%)	0.43
Calcium hydrogen phosphate	1.83	Lysine (%)	1.03
Sodium chloride	0.30	Methionine (%)	0.41
DL-Methionine (98%)	0.10		
L-Lysine HCL (78%)	0.10		
Vitamin premix[1]	0.03		
Mineral premix[2]	0.20		
Choline chloride (50%)	0.20		
Ethoxyquin (33%)	0.03		
Total	100.00		

[1] Vitamin premix provided the following per kg of diets: vitamin A (retinylacetate), 12500 IU; vitamin D3(cholecalciferol),2 500 IU; vitamin E (DL-a-tocopherol acetate),18.75 mg; vitamin K3 (menadione sodium bisulfate), 2.65 mg; VB_1 2 mg; VB_2 6 mg; VB_6 6 mg; vitamin B12 (cyanocobalamin), 0.025 mg; biotin,0.0325 mg; folic acid,1.25 mg; pantothenic acid,1.25 mg; nicotinic acid,50mg. [2] Mineral premix provided per kilogram of complete diet: Cu (as copper sulfate)8 mg, Zn (as zinc sulfate)75 mg, Fe (as ferrous sulfate)80 mg, Mn (as manganese sulfate)100 mg, Se (as sodium selenite)0.15 mg, I (as potassium iodide)0.35 mg. [3]Calculated value based on the analyzed data of experimental diets.

Table 2 Sequences of the oligonucleotide primers used for quantitative real-time PCR for immune-related genes expreesion[1]

Genes	Forward Primer sequence (5′→ 3′)	Reverse Primer sequence (5′→ 3′)	GenBank Accession No.	PCR size (bp)
TLR4	F:CCACTATTCGGTTGGTGGAC	R:ACAGCTTCTCAGCAGGCAAT	NM_001030693.1	120
MyD88	F:TGCAAGACCATGAAGAACGA	R:TCACGGCAGCAAGAGAGATT	NM_001030962.3	123
NF-kB	F:TGGAGAAGGCTATGCAGCTT	R:CATCCTGGACAGCAGTGAGA	NM_205134.1	117
IL-1β	F:TCATCTTCTACCGCCTGGAC	R:GTAGGTGGCGATGTTGACCT	NM_204524.1	149
IL-6	F:GATCCGGCAGATGGTGATAA	R:AGGATGAGGTGCATGGTGAT	NM_204628.1	126
IL-8	F-GGCTTGCTAGGGGAAATGA	R-AGCTGACTCTGACTAGGAAACTGT	NM_205498.1	200
TNF-SF15	F-CCCCTACCCTGTCCCACAA	R-TGAGTACTGCGGAGGGTTCAT	NM_204267.1	67
IFN-γ	F:CTTCCTGATGGCGTGAAGA	R:GAGGATCCACCAGCTTCTGT	NM_205149.1	127
TGβ4	F:AGAGCATTGCCAAGAAGCAC	R:GCAGTAGTCGGTGTCGAGGT	NM_001318456.1	119
A20	F:GAGAACGCAGAGCCTACACC	R:CCAACCTTCTTCCTGCACAT	NM_001277522.1	95
Tollip	F:CATGGTACCTGTGGCAATACC	R:GCACTGAGCGGATTACTTCC	NM_001006471	122
PI3K	F:AACATCTGGCAAAACCAAGG	R:CTGCAATGCTCCCTTTAAGC	NM001004410	150
PIgR	F:ATGAAGCAGAGCCAGGAGAC	R:GAGTAGGCGAGGTCAGCATC	NM001044644.1	128
MLCK	F:TTGACATGGAGGTTGTGGAA	R:GAAGTGACGGGACTCCTTGA	NM_001322361.1	119
Claudin1	F:AAGTGCATGGAGGATGACCA	R:GCCACTCTGTTGCCATACCA	NM_001013611.2	119
Occludin	F:AGTTCGACACCGACCTGAAG	R:TCCTGGTATTGAGGGCTGTC	NM_205128.1	124
ZO-1	F:ACAGCTCATCACAGCCTCCT	R:TGAAGGGCTTACAGGAATGG	XM_015278981.1	125
ZO-2	F:CACCACCACCTGTTTCTGTG	R:TTCACTCCCTTCCTCTTCCA	NM_204918.1	119

[1]Primers were designed and synthesized by Sango Biotech (Shanghai) Co., Ltd.

F: forward; R: reverse; IFN-γ =interferon-γ; IL =interleukin; LYZ=lysozyme; TLR= Toll-like receptor; TNFSF15= Tumor necrosis factor super family 15.

Table 3 Effect of dietary *Enterococcus faecium* NCIMB 11181 supplementation on broiler chicken performance challenged with *Salmonella Typhimurium* (ST) (n=8).

Dosage (g/T)	Treatment[1]	Body weight (g/bird)		Body weight gain (g/bird)	
		d11	21d	1-11d	12-21d
0	-	182	500	144	318
200	-	193	530	154	337
0	+	183	482	144	298
200	+	176	458	138	281
SEM[2]		2.78	9.87	2.73	7.60
Main factors					
0		183	491	144	308
200		185	494	146	309
Non-challenged		188	515[a]	149	327[a]
Challenged		180	470[b]	141	290[b]
Main factors and Interaction (P Value)[3]					
Enterococcus faecium		0.697	0.819	0.694	0.905
Challenged		0.161	0.011	0.176	0.006
Enterococcus faecium × Challenged		0.108	0.082	0.136	0.106

[1]Challenged with *Salmonella Typhimurium*; -, without ST challenged; +, with ST challenged

[2]SEM, standard error of the mean

[3]P-value represent the main effect of the diet, the main effect of ST challenged, and the interaction between the dietary treatments and ST challenged

[4a,b] Means in the same column without common superscripts differ significantly ($P < 0.05$).

Table 4 Effect of dietary *Enterococcus faecium* NCIMB 11181 supplementation on intestinal morphology, histopathological scores and intestinal cells proliferation and apoptosis index (OD) of broiler chickens challenged with *Salmonella Typhimurium* (ST).

Dosage (g/T)	Treatment[1]	Villous height (μm)	Crypt depth (μm)	V/C	HE scores	Tunel
0	-	899.2	134.5	6.81	1.46	7767
200	-	957.7	145.4	6.59	1.00	3514
0	+	743.4	133.2	5.58	4.00	7538
200	+	832.2	142.6	5.85	3.40	5391
SEM[2]		43.27	5.92	0.293	0.334	1275.7
Main factors						
0		821.7[a]	133.9	6.18	2.81[a]	7652[a]
200		895.3[b]	144.0	6.22	2.09[b]	4452[b]
Non-challenged		928.3[a]	140.5	6.70[a]	1.24[b]	5641[b]
Challenged		788.7[b]	137.8	5.71[b]	3.70[a]	6464[a]
Main factors and Interaction (P Value)[3]						
Enterococcus faecium		0.042	0.498	0.537	0.047	0.016
Challenged		0.001	0.251	0.048	0.000	0.037
Enterococcus faecium × Challenged		0.298	0.497	0.264	0.392	0.057

[1]Challenged with *Salmonella Typhimurium*; -, without ST challenged; +, with ST challenged

[2]SEM, standard error of the mean

[3]P-value represent the main effect of the diet, the main effect of ST challenged, and the interaction between the dietary treatments and ST challenged

[4a,b]Means in the same column without common superscripts differ significantly ($P < 0.05$).

Table 5 Effect of dietary *Enterococcus faecium* NCIMB 11181 supplementation on the number of *Salmonella* in the liver, spleen and ceca of chicks (the recovery of *Salmonella* from internal organs (liver, spleen, cecum) of broilers chickens (n = 15).

Dosage (g/T)	Treatment[1]	Salmonella recovery[5]				
		liver		spleen		cecum
		CFU/g[4]	No. positive[6]	CFU/g4	No. positive[6]	
0	-	0.00	0/30	0.00	0/30	0.00 [c]
200	-	0.00	0/30	0.00	0/30	0.00[c]
0	+	0.74	14/29	0.29	7/29	6.85[a]
200	+	0.29	6/29	0.22	5/29	5.32[b]
SEM[2]		0.125		0.089		0.146
Main factors[2]						
0		0.37		0.14		3.43
200		0.14		0.11		2.66
Non-challenged		0.00[b]		0.00		0.00
Challenged		0.52[a]		0.25		6.09
Main factors and Interaction (P Value)[3]						
Enterococcus faecium		0.350		0.842		0.042
Challenged		0.038		0.171		< 0.001
Enterococcus faecium × Challenged		0.350		0.842		0.031

[1]Challenged with *Salmonella Typhimurium*; -, without ST challenged; +, with ST challenged

[2]SEM, standard error of the mean

[3]P-value represent the main effect of the diet, the main effect of ST challenged, and the interaction between the dietary treatments and ST challenged

4CFU/g recovered from tissues expressed in log10 values

5Challenge strain recovery by direct and enrichment cultures of liver, spleen, and ceca of birds.

[6]Number of positive samples per total number of samples after bacterial recovery

[a,b] Mean±SD in the same column without common superscripts differ significantly (P < 0.05).

Table 6 Effect of dietary *Enterococcus faecium* NCIMB 11181 supplementation on immune-related gene expression in caecum tonsil of broilers challenged with *Salmonella Typhimurium* (n = 8).

Dosage (g/T)	Treatment [1]	*TLR4*	*MyD88*	*NF-κB*	*IL-1β*	*IL-6*	*IL-8*	*TNF-α*	*IFN-γ*	*TGβ4*	*plgR*	*A20*	*Tollip*	*PI3K*
0	-	1.03	1.07	1.02	1.07	1.12	1.08	1.02	1.02	1.02	1.04	1.01	1.03	1.02
200	-	1.00	0.94	1.09	1.38	0.75	1.93	1.01	1.52	0.80	1.28	1.08	1.21	1.16
0	+	1.33	1.23	1.19	1.85	1.14	1.96	1.13	2.61	0.77	1.60	1.11	1.26	1.56
200	+	1.31	1.47	1.33	2.03	0.82	1.88	1.50	2.20	0.76	2.39	1.05	1.65	1.44
SEM		0.086	0.074	0.047	0.214	0.149	0.287	0.090	0.184	0.084	0.125	0.032	0.070	0.083
Main factors [2]														
0		1.15	1.15	1.11	1.46	1.13	1.52	1.08	1.82	0.90	1.23b	1.06	1.15b	1.29
200		1.16	1.20	1.21	1.71	0.78	1.91	1.26	1.83	0.78	1.71a	1.06	1.43a	1.30
Non-challenged		1.02	1.01b	1.05b	1.23	0.94	1.50	1.02	1.27b	0.92	1.16b	1.04	1.12b	1.09[b]
Challenged		1.32	1.35a	1.26a	1.94	0.98	1.92	1.32	2.43a	0.77	1.99a	1.08	1.46a	1.50[a]
Main factors and Interaction (P Value) [3]														
Enterococcus faecium		0.910	0.699	0.237	0.573	0.279	0.523	0.296	0.883	0.516	0.049	0.954	0.019	0.950
Challenged		0.099	0.017	0.024	0.110	0.898	0.493	0.096	0.001	0.417	0.004	0.572	0.006	0.014
Enterococcus faecium × Challenged		0.975	0.170	0.714	0.882	0.940	0.447	0.282	0.116	0.552	0.520	0.320	0.339	0.426

[1]Challenged with *Salmonella Typhimurium*; -, without ST challenged; +, with ST challenged

[2]SEM, standard error of the mean

[3]P-value represent the main effect of the diet, the main effect of ST challenged, and the interaction between the dietary treatments and ST challenged

[4]a,b Means in the same column without common superscripts differ significantly ($P < 0.05$).

Table 7 Effect of dietary *Enterococcus faecium* NCIMB 11181 supplementation on mRNA abundance of tight junction (gene expression) in the jejunum of broiler chickens challenged with *Salmonella Typhimurium* (n = 8).

Dosage (g/T)	Treatment[1]	*MLCK*	*Claudin-1*	*Occludin*	*ZO-1*	*ZO-2*
0	-	1.03	1.05	1.06	1.02	1.01
200	-	0.85	0.86	0.74	0.98	1.04
0	+	1.61	0.48	0.47	0.69	0.68
200	+	2.03	0.58	0.59	0.74	0.66
SEM		0.153	0.070	0.074	0.052	0.054
Main factors[2]						
0		1.30	0.79	0.79	0.87	0.86
200		1.44	0.73	0.68	0.86	0.85
Non-challenged		0.94[b]	0.95[a]	0.90[a]	1.00[a]	1.02[a]
Challenged		1.84[a]	0.53[b]	0.52[b]	0.72[b]	0.67[b]
Main factors and Interaction (P Value)[3]						
Enterococcus faecium		0.638	0.682	0.419	0.981	0.971
Challenged		0.002	0.001	0.007	0.005	0.000
Enterococcus faecium × Challenged		0.233	0.197	0.082	0.660	0.763

[1]Challenged with *Salmonella Typhimurium*; -, without ST challenged; +, with ST challenged

[2]SEM, standard error of the mean

[3]P-value represent the main effect of the diet, the main effect of ST challenged, and the interaction between the dietary treatments and ST challenged

[4a,b] Means in the same column without common superscripts differ significantly ($P < 0.05$).

Table 8 Effect of dietary *Enterococcus faecium* NCIMB 11181 supplementation on anti-*Salmonella* specific IgA (**OD_{450nm}**) of jejunum mucosa and serum anti-*Salmonella* IgG (**OD_{450nm}**) of broiler chicken challenged with *Salmonella Typhimurium* (n=8).

Dosage (g/T)	Treatment[1]	IgA	IgG
0	-	0.07[c]	0.45
200	-	0.12[c]	0.49
0	+	0.33[b]	1.05
200	+	0.48[a]	0.84
SEM		0.015	0.066
Main factors[2]			
0		0.20	0.75
200		0.30	0.66
Non-challenged		0.10	0.47[b]
Challenged		0.41	0.94[a]
Main factors and Interaction (P Value)[3]			
Enterococcus faecium		0.047	0.340
Challenged		0.009	< 0.001
Enterococcus faecium × Challenged		0.039	0.170

[1]Challenged with *Salmonella Typhimurium*; -, without ST challenged; +, with ST challenged

[2]SEM, standard error of the mean

[3]P-value represent the main effect of the diet, the main effect of ST challenged, and the interaction between the dietary treatments and ST challenged

[4a,b] Means in the same column without common superscripts differ significantly ($P < 0.05$).

[5]All values represent the means with subtracted background OD_{450nm}. Serum were diluted at 1:20, and intestinal mucosa was diluted at 1:60 for the *Salmonella* antigen detection, respectively, using ELISAs. Each sample was assayed in duplicate.

Table 9 Effect of dietary *Enterococcus faecium* NCIMB 11181 supplementation on α-diversity of cecal microbiota of broiler chickens challenged with *Salmonella Typhimurium* (n=8)

Dosage (g/T)	Treatment	Clean reads/tags	Effective reads /tags	OTUs	Chao1	goods-coverage	Shannon	Simpson
0	-	141844	130059	5982	5894.15	0.98	6.41	0.92
200	-	157548	139395	7020	7289.19	0.98	6.76	0.93
0	+	139543	125817	6338	6173.16	0.98	6.74	0.92
200	+	159097	146880	6425	6524.50	0.98	6.76	0.94
SEM		20771	17075	1864	429.555	0.001	0.121	0.006
Main factors								
0		140694	127938	6160	6033.65	0.98	6.57	0.92
200		158318	143138	6723	6906.84	0.98	6.76	0.94
Non-challenged		149696	134727	6501	6591.67	0.98	6.58	0.93
Challenged		149320	136349	6382	6348.83	0.98	6.75	0.93
Main factors and Interaction (P Value)								
Enterococcus faecium		0.044	0.030	0.482	0.339	0.234	0.472	0.178
Challenged		0.964	0.806	0.892	0.788	0.713	0.509	0.942
Enterococcus faecium × Challenged		0.816	0.379	0.550	0.565	0.898	0.514	0.775

[1]Challenged with *Salmonella Typhimurium*; -, without ST challenged; +, with ST challenged

[2]SEM, standard error of the mean

[3]P-value represent the main effect of the diet, the main effect of ST challenged, and the interaction between the dietary treatments and ST challenged