**Supplementary materials**

**Table S1** Composition and nutrient levels of the basal diets

|  |  |  |
| --- | --- | --- |
| Items (%) | Day 1-21 | Day 22-42 |
| Ingredients |  |  |
| Corn | 56.44 | 55.90 |
| Soybean meal(43% CP） | 32.11 | 31.49 |
| Dicalcium phosphate | 1.64 | 1.32 |
| Limestone | 1.40 | 1.32 |
| NaCl | 0.30 | 0.30 |
| DL-Met (98%) | 0.21 | 0.10 |
| Multimineral \* | 0.15 | 0.15 |
| Multivitamin † | 0.15 | 0.15 |
| Choline chloride (60%) | 0.10 | 0.10 |
| Soybean oil | 3.16 | 4.94 |
| Phytase (10000 IU/g ) | 0.01 | 0.01 |
| L-Lys-H2SO4 (70%) | 0.11 | 0.00 |
| Zeolite powder | 0.22 | 0.22 |
| corn gluten meal | 4.00 | 4.00 |
| SUM | 100.00 | 100.00 |
|  |  |  |
| Nutrient and energy concentration ‡ |  |  |
| Metabolisable energy（kcal/kg） | 2950 | 3050 |
| Crude protein | 21 | 20 |
| Ca | 1.0 | 0.9 |
| P | 0.64 | 0.58 |
| STTD P | 0.46 | 0.42 |
| Lys | 1.1 | 1.0 |
| Met | 0.89 | 0.77 |
| Thr | 0.78 | 0.76 |
| Trp | 0.22 | 0.22 |

\* Supplied the following per kg complete feed: Cu, 8 mg; Zn, 75 mg; Fe, 80 mg; Mn, 100 mg; Se, 0·15 mg; I, 0·35 mg.

† Supplied the following per kg complete feed: retinyl acetate, 24 mg; cholecalciferol, 6 mg; menadione, 2·65 mg; thiamine, 2 mg; riboflavin, 6 mg; cyanocobalamin, 0·025 mg; α-tocopheryl acetate, 20 mg; biotin, 0·0325 mg; folic acid, 1·25 mg; pantothenic acid, 12 mg; niacin, 50 mg.

‡ Calculated value.

**Table S2** Primers used for quantitative real-time PCR

|  |  |
| --- | --- |
| Target genes | Primer sequence (5' to 3') |
| *TNF-α* | F:ATGAACCCTCCGCAGTACTC |
| R:AAGAGGCCACCACACGACA |
| *IL-1β* | F:CCTGTCTCTGTCCCTACCCCCTA |
| R:GTCAACGGGTGTGCTGCAGGAAC |
| *IL-8* | F:CCAAGCACACCTCTCTTCCA |
| R:GCAAGGTAGGACGCTGGTAA |
| *TGF-β* | F:ATGTGTTCCGCTTTAACGTGTC |
| R:GCTGCTTTGCTATATGCTCATC |
| *IFN-γ* | F:GCTCCCGATGAACGACTTGA |
| R:TGTAAGATGCTGAAGAGTTCATTCG |
| *TLR2* | F:GGGGCTCACAGGCAAAATC |
| R:AGCAGGGTTCTCAGGTTCACA |
| *TLR4* | F:AGTCTGAAATTGCTGAGCTCAAAT |
| R:GCGACGTTAAGCCATGGAAG |
| *MyD88* | F:GAAGTTGGGCCACGACTACCT |
| R:TTGCACTTGACCGGAATCAGC |
| *NF-κB* | F:TGACCGCCAATAGCTTGTCC |
| R:ACAGCTAAATGCAATGCCGTTC |
| *JAK1* | F:TGCACCGTGACTTAGCAGCAAG |
| R:TCTGAATCAAGCATTCTGGAGCATACC |
| *JAK2* | F:TCGCTATGGCATTATTCG |
| R:GTGGGGTTTGGTCCTTTT |
| *JAK3* | F:GCATCCGCCGCCGTGTTG |
| R:AGCACCGCAGCCTCTCCAG |
| *STAT1* | F:TAAAGAGGGAGCAATCAC |
| R:ATCAGGGAAAGTAACAGC |
| *STAT6* | F:GCAACCTCTACCCCAACA |
| R:TCCCTTTCGCTTTCCACT |
| *iNOs* | F:TGGGTGGAAGCCGAAATA |
| R:GTACCAGCCGTTGAAAGGAC |
| *ZO-1* | F:CTTCAGGTGTTTCTCTTCCTCCTC |
| R:CTGTGGTTTCATGGCTGGATC |
| *Occludin* | F:ACGGCAGCACCTACCTCAA |
| R:GGGCGAAGAAGCAGATGAG |
| *Claudin-2* | F:CTGCTCACCCTCATTGGA |
| R:AACTCACTCTTGGGCTTCTG |
| *β-actin* | F:AGACATCAGGGTGTGATGGTTGGT |
| R:TGGTGACAATACCGTGTTCAATGG |

F, forward; R, reverse. *TNF-α*, *tumor necrosis factor alpha*; *IL-1β*, *interleukin 1 beta*; *IL-8*, *interleukin 8*; *TGF-β*, *transforming growth factor beta*; *IFN-γ*, *interferon gamma*; *TLR-2*, *toll-like receptor 2*; *TLR-4*, *toll-like receptor 4*; *MyD88*, *myeloiddifferentiationfactor88*; *NF-κB*, *nuclear factor kappa B*; *JAK1*, *Janus kinase 1*; *JAK2*, *Janus* *kinase 2*; *JAK3*, *janus kinase 3*; *STAT1*, *signal transducers and activators of transcription 1*; *STAT6*, *signal transducers and activators of transcription 6*; *iNOS*, *inducible nitric oxide synthase*; *ZO-1*, *zonula occludens 1*.

**Table S3** Good's coverage estimators of *C. perfringens* challenge and dietary EA levels treatments

|  |  |  |  |
| --- | --- | --- | --- |
| Dietary EA levels | 0 mg/kg | 500 mg/kg | 　 |
| *C. perfringens* challenge | - | + | - | + | SEM |
| Good's coverage | 0.9976 | 0.9980 | 0.9976 | 0.9979 | 0.00007766 |

All values are expressed as the means (n=5 in EAXCP Group, n = 6 in the other three groups).

**Table S4** Effect of *C. perfringens* challenge and dietary EA levels on alpha diversity of cecal microbiota

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Dietary EA levels | 0 mg/kg | 500 mg/kg | 　 | *P*-values |
| *C. perfringens* challenge | - | + | - | + | SEM | *C. perfringens* challenge | Dietary EA level | Interaction |
| Observed species | 715.33 | 728.33 | 780.00 | 762.20 | 12.052 | 0.9182 | 0.0459\* | 0.5123 |
| Shannon | 5.77 | 5.87 | 5.96 | 6.39 | 0.099 | 0.2241 | 0.0465\* | 0.4608 |
| Chao1 | 1200.58 | 987.58 | 1043.61 | 996.84 | 65.301 | 0.3489 | 0.5914 | 0.5462 |
| Simpson | 0.94 | 0.94 | 0.93 | 0.96 | 0.005 | 0.1841 | 0.4753 | 0.0836 |
| ACE | 813.03 | 802.70 | 870.52 | 836.73 | 14.425 | 0.4505 | 0.1265 | 0.6866 |

All values are expressed as the means (n=5 in EAXCP Group, n = 6 in the other three groups).\* Significant main effect (*P*<0.05) of *C. perfringens* challenge or dietary EA level.



**Fig. S1** Effect of *C. perfringens* challenge and dietary EA levels on relative mRNA expression of jejunal inflammation-related pathway and cytokine genes in jejunal mucosa of broilers at day 42. (**A** and **B**) The relative mRNA expressions of *transforming growth factor-β* (*TGF-β*) and *interferon γ* (*IFN-γ*). (C) The relative mRNA expression of *myeloiddifferentiationfactor88* (*MyD88*). (**D**, **E**, and **F)** The relative mRNA expression of *Janus kinase 1* (*JAK1*), *Janus* *kinase 2* (*JAK2*), and *signal transducers and activators of transcription 1* (*STAT1*). Unchallenged, birds without *C. perfringens* infection; challenged, birds with *C. perfringens* infection. Values are means (n = 6) with their standard errors represented by vertical bars.



**Fig. S2** The quality of sequencing data and beta diversity of cecal microbiota. (**A**) Venn diagram of the OTUs. (**B**) The rarefaction curve analysis of the microbial species. (**C** and **D**) Box plot and principal co-ordinates analysis (PCoA) plot of beta diversity. All values are expressed as the means (n=5 in EAXCP Group, n = 6 in the other three groups).



**Fig.S3** The most abundant (top 10) phyla and genus of cecal microbiota. (**A**) The most abundant (top 10) phyla of cecal microbiota. (**B**) The most abundant (top 10) genus of cecal microbiota. (**C**, **D**, **E**, **F**, and **G**) The relative abundance of *Firmicutes*, *Desulfobacterota*, *Campilobacterota*, *Elusimicrobia*, and *[Ruminococcus]\_ torques\_group*.