*Supplementary Material*

**Table S1.** Effect of *E. faecium* on alpha diversity of microbial community in colonic content of piglets challenged with ETEC K88

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | -ETEC |  | +ETEC |  | *P*-value |
|  | CON | PRO |  | CON | PRO |  | PRO | ETEC | PRO×ETEC |
| Observed-species | 505.17±24.98 | 502.50±21.53 |  | 482.83±25.60 | 493.83±24.93 |  | 0.87 | 0.53 | 0.78 |
| Shannon | 5.97±0.16 | 5.73±0.24 |  | 5.90±0.21 | 5.59±0.18 |  | 0.19 | 0.62 | 0.86 |
| Chao1 | 541.10±27.70 | 530.41±19.46 |  | 514.85±25.45 | 536.56±23.34 |  | 0.82 | 0.68 | 0.51 |
| PD whole tree | 31.40±1.58 | 31.42±1.09 |  | 29.58±1.43 | 30.28±1.37 |  | 0.79 | 0.29 | 0.81 |

Data are presented as means ± SE (*n* = 6).

-ETEC, infusing the essential medium; +ETEC, infusing the *Escherichia coli*; CON, control group; PRO, *Enterococcus faecium*-supplemented group.

**Table S2.** Effect of *E. faecium* on the relative abundance for the top 30 most abundant genera in the colon of piglets challenged with ETEC K88

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | -ETEC |  | +ETEC |  | *P*-value |
|  | CON | PRO |  | CON | PRO |  | PRO | ETEC | PRO×ETEC |
| *Fusobacterium* | 10.91±4.57 | 8.81±5.36 |  | 14.68±5.04 | 8.96±6.95 |  | 0.11 | 0.97 | 0.64 |
| *Escherichia-Shigella* | 9.99±3.86ab | 8.38±2.03b |  | 15.38±1.26a | 14.45±3.10ab |  | 0.69 | 0.02 | 0.84 |
| *Prevotella\_2* | 7.38±2.13 | 7.74±2.92 |  | 14.06±3.46 | 4.88±1.07 |  | 0.32 | 0.29 | 0.42 |
| *Bacteroides* | 8.14±1.97 | 7.61±3.12 |  | 5.19±1.19 | 5.14±2.01 |  | 0.40 | 0.34 | 0.80 |
| *Alloprevotella* | 5.99±1.67 | 4.88±1.04 |  | 7.08±1.99 | 4.04±0.96 |  | 0.32 | 0.82 | 0.48 |
| *Butyricimonas* | 1.66±0.44 | 1.81±0.52 |  | 4.44±1.32 | 1.68±0.41 |  | 0.18 | 0.14 | 0.10 |
| *Rikenellaceae\_RC9\_gut\_group* | 3.49±0.85 | 4.41±0.77 |  | 3.49±0.74 | 4.14±1.42 |  | 0.46 | 0.70 | 0.58 |
| *Lachnoclostridium* | 4.86±1.23a | 2.63±0.46ab |  | 1.99±0.45b | 2.23±0.68b |  | 0.26 | 0.02 | 0.28 |
| *Prevotellaceae\_UCG-003* | 1.24±1.11 | 1.49±1.25 |  | 0.57±0.24 | 0.80±0.42 |  | 0.87 | 0.56 | 0.89 |
| *Ruminococcaceae\_UCG-002* | 1.90±0.95 | 1.55±0.66 |  | 1.44±0.52 | 1.44±0.65 |  | 0.95 | 0.83 | 0.92 |
| *Prevotellaceae\_NK3B31\_group* | 1.30±0.88b | 0.74±0.16ab |  | 1.76±0.23a | 1.70±0.79ab |  | 0.95 | 0.05 | 0.32 |
| *Actinobacillus* | 2.49±0.96 | 1.17±0.21 |  | 0.97±0.39 | 0.86±0.37 |  | 0.89 | 0.29 | 0.63 |
| *Tyzzerella* | 0.14±0.07 | 0.22±0.10 |  | 1.50±0.90 | 0.15±0.08 |  | 0.57 | 0.57 | 0.31 |
| *Parabacteroides* | 3.13±0.45 | 1.91±0.61 |  | 1.86±0.42 | 1.39±0.41 |  | 0.06 | 0.10 | 0.63 |
| *Alistipes* | 1.47±0.62 | 1.23±0.49 |  | 1.60±0.51 | 0.76±0.21 |  | 0.17 | 0.68 | 0.32 |
| *Eubacterium\_coprostanoligenes\_group* | 0.91±0.54 | 0.77±0.47 |  | 1.08±0.66 | 0.52±0.23 |  | 0.91 | 0.95 | 0.77 |
| *p-1088-a5\_gut\_group* | 0.04±0.02 | 0.69±0.64 |  | 0.06±0.04 | 0.12±0.07 |  | 0.26 | 0.12 | 0.70 |
| *Ruminococcaceae\_NK4A214\_group* | 1.75±0.53 | 1.35±0.13 |  | 1.04±0.35 | 1.05±0.36 |  | 0.89 | 0.07 | 0.97 |
| *Pasteurella* | 0.73±0.49 | 0.59±0.46 |  | 0.18±0.07 | 0.20±0.10 |  | 0.99 | 0.35 | 0.85 |
| *Lactobacillus* | 1.44±0.33 | 1.30±0.35 |  | 0.44±0.22 | 1.44±0.44 |  | 0.16 | 0.08 | 0.11 |
| *Anaerotruncus* | 0.40±0.13 | 0.14±0.03 |  | 0.55±0.42 | 0.12±0.03 |  | 0.15 | 0.45 | 0.64 |
| *Oscillospira* | 0.69±0.17 | 0.73±0.15 |  | 0.76±0.13 | 0.75±0.36 |  | 0.52 | 0.73 | 0.39 |
| *unidentified\_Ruminococcaceae* | 0.78±0.32 | 0.45±0.14 |  | 0.40±0.14 | 0.76±0.36 |  | 0.92 | 0.66 | 0.23 |
| *Bilophila* | 0.87±0.33a | 0.17±0.05b |  | 0.27±0.09ab | 0.24±0.08b |  | 0.04 | 0.29 | 0.22 |
| *Desulfovibrio* | 0.89±0.28 | 0.90±0.21 |  | 0.74±0.16 | 0.93±0.13 |  | 0.37 | 0.93 | 0.57 |
| *Eisenbergiella* | 0.23±0.05 | 0.21±0.09 |  | 0.34±0.13 | 0.58±0.33 |  | 0.80 | 0.44 | 0.49 |
| *Odoribacter* | 0.26±0.12 | 0.09±0.05 |  | 0.62±0.33 | 0.24±0.07 |  | 0.28 | 0.15 | 0.41 |
| *Ruminococcaceae\_UCG-005* | 0.21±0.07 | 0.47±0.30 |  | 0.38±0.18 | 0.40±0.19 |  | 0.74 | 0.87 | 0.86 |
| *Victivallis* | 0.44±0.30 | 0.20±0.09 |  | 0.03±0.01 | 0.22±0.13 |  | 0.19 | 0.11 | 0.25 |
| *Prevotella\_7* | 0.04±0.03 | 0.37±0.28 |  | 0.00±0.00 | 0.03±0.03 |  | 0.18 | 0.08 | 0.35 |

Data are presented as means ± SE (*n* = 6).

-ETEC, infusing the essential medium; +ETEC, infusing the *Escherichia coli*; CON, control group; PRO, *Enterococcus faecium*-supplemented group.

a, b Means within a row with different superscripts are significantly different (*P* < 0.05)

**Table S3.** Effect of *E. faecium* on short chain fatty acid concentrations of piglets challenged with ETEC K88

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | -ETEC |  | +ETEC |  | *P*-value |
|  | CON | PRO |  | CON | PRO |  | PRO | ETEC | PRO×ETEC |
| Acetate, mg/g | 0.61±0.07 | 0.59±0.05 |  | 0.73±0.09 | 0.61±0.12 |  | 0.39 | 0.39 | 0.58 |
| Propionic acid, mg/g | 0.25±0.03 | 0.23±0.04 |  | 0.33±0.05 | 0.21±0.04 |  | 0.15 | 0.27 | 0.32 |
| Butyric acid, mg/g | 0.11±0.02 | 0.09±0.02 |  | 0.14±0.02 | 0.09±0.03 |  | 0.20 | 0.27 | 0.71 |

Data are presented as means ± SE (*n* = 8).

-ETEC, infusing the essential medium; +ETEC, infusing the *Escherichia coli*; CON, control group; PRO, *Enterococcus faecium*-supplemented group.

**Figure S1.** The experimental design of the different treatments and procedures.



Day 1-7: treatment with *E. faecium* for one week

Day 8: ETEC challenge only at day 8

Day 10: tissue sample collection

**Figure S2.** Comparison of the gut microbiota composition among four groups. Principal coordinate analysis to visualize the unweighted UniFrac distances of colon digesta samples from individual piglet.

C.ETEC: CON+ETEC; C: CON-ETEC; P.ETEC: PRO+ETEC; P: PRO-ETEC.

