**Additional file (Table S1-S4; Fig. S1-S2)**

**Low-protein diets supplemented with casein hydrolysate favor the microbiota and enhance the mucosal humoral immunity in the colon of pigs**

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**Table S1** List of primers used in the present study.

|  |  |  |  |
| --- | --- | --- | --- |
| Target gene | Primer sequences (5'→3') | Reference | Annealing temperature, °C |
| *TNF-α* | F:CCACGCTCTTCTGCCTACTGCR:GCTGTCCCTCGGCTTTGAC | [1] | 60 |
| *IL-1β* | F:AGTGGAGAAGCCGATGAAGAR:CATTGCACGTTTCAAGGATG | [2] | 60 |
| *IFN-γ* | F:TCCAGCGCAAAGCCATCAGTGR:ATGCTCTCTGGCCTTGGAACATAGT | [3] | 60 |
| *IL-2* | F:AGCTCTGGAGGGAGTGCTAAR:TGTTTCAGATCCCTTTAGTTCCA | [4] | 60 |
| *IL-4* | F:GCTGCCCCAGAGAACACGACR:AGGTTCCTGTCAAGTCCGCTC | [4] | 60 |
| *IL-10* | F:GTCCGACTCAACGAAGAAGGR:GCCAGGAAGATCAGGCAATA | [5] | 60 |
| *TGF-β* | F:GAAGATGCTTGGAGCTGAGGR:TGGGACTTTGTCTTGGGAAC | [5] | 60 |
| *IL-12 p40* | F:GGAGTATAAGAAGTACAGAGTGGR: GATGTCCCTGATGAAGAAGC | [6] | 60 |
| *IL-18* | F:AGGGACATCAAGCCGTGTTTR:CGGTCTGAGGTGCATTATCTGA | [7] | 60 |
| *IL-5* | F:TGCCTACGTTAGTGCCATTGR:TGCCTACGTTAGTGCCATTG | [8] | 60 |
| *IL-6* | F:TGGCTACTGCCTTCCCTACCR:CAGAGATTTTGCCGAGGATG | [6] | 60 |
| *IL-13* | F:AAGTGGCCCAGTTCGTAAAAGAR:ACCCGTGGCGAAAAATCA | [7] | 60 |
| β-actin | F:AGAGCGCAAGTACTCCGTGTR: ACATCTGCTGGAAGGTGGAC | [5] | 60 |
| *GPR43* | F:GTACCTGCCTGGGATCGTCTR:TGACCACCATGGGGATGAAG | [9] | 60 |
| *GPR41* | F:CTCATCACCAGCTACTGCCGR:AATTCAGGGTGCTGAGGAGC | [10] | 60 |
| *TLR2* | F:TCACTTGTCTAACTTATCATCCTCTTGR:TCAGCGAAGGTGTCATTATTGC | [6] | 60 |
| *TLR4* | F:GCCATCGCTGCTAACATCATCR:CTCATACTCAAAGATACACCATCGG | [6] | 60 |
| *NOD1* | F:ACCGATCCAGTGAGCAGATAR:AAGTCCACCAGCTCCATGA | [6] | 60 |
| *NOD2* | F:CCTTTTGAAGATGCTGCCTGR:GATTCTCTGCCCCATCGTAG | [6] | 60 |
| *NF-kB* | F:CTCGCACAAGGAGACATGAAR:ACTCAGCCGGAAGGCATTAT | [6] | 60 |
| *MAPK* | F:TGCAAGGTCTCTGGAGGAATR:CTGAACGTGGTCATCCGTAA | [11] | 60 |
| *ZO-1* | F:*GAGGATGGTCACACCGTGGT*R:GGAGGATGCTGTTGTCTCGG | [1] | 60 |
| Occludin | F:ATGCTTTCTCAGCCAGCGTAR:AAG GTTCCATAGCCTCGGTC | [1] | 60 |
| *DEFB-1* | F:ACCGCCTCCTCCTTGTATTCR:GGTGCCGATCTGTTTCATCT | [6] | 60 |
| *DEFB-2* | F:CTGTCTGCCTCCTCTCTTCCR:CAGGTCCCTTCAATCCTGTT | [6] | 60 |
| *MUC-2* | F:CTGCTCCGGGTCCTGTGGGAR:CCCGCTGGCTGGTGCGATAC | [5] | 60 |
| *MUC-4* | F:GTGCCTTGGGTGAGAGGTTAR:CACTCTGCCGTTCTTTCC | [12] | 60 |

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**Table S2** The growth performance of pigs.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Items | Control | LPA | LPC | *P*-value |
| Average daily gain (ADG),kg/d | 0.55 ± 0.02a | 0.52 ± 0.02a | 0.62 ± 0.01b | 0.020 |
| Average daily feed intake (ADFI), kg/d |  1.24 ± 0.05ab | 1.18 ± 0.04a | 1.35 ± 0.05b | 0.027 |
| Feed:gain (F:G),kg/kg | 2.35 ± 0.07 | 2.29 ± 0.07 | 2.33 ± 0.09 | 0.790 |

Values are means ± SEMs (n = 7). LPA: Low-protein diets supplemented with free amino acids. LPC: Low-protein diets supplemented with casein hydrolysate.

**Table S3** The pH of colonic digesta and the diversity estimation of 16S rRNA gene libraries from microbiota in the colonic digesta.

|  |  |  |
| --- | --- | --- |
| Groups | pH of colonic digesta | Richness and diversity estimator |
| Chao1 | Shannon |
| Control | 6.81 ± 0.19a | 226.75 ± 10.76a | 4.11 ± 0.20a |
| LPA | 6.75 ± 0.14a | 285.73 ± 6.69b | 5.73 ± 0.07b |
| LPC | 6.31 ± 0.07b | 243.37 ± 19.02ab | 4.72 ± 0.24a |

Values are means ± SEMs (n = 7). LPA: Low-protein diets supplemented with free amino acids. LPC: Low-protein diets supplemented with casein hydrolysate.

**Table S4** The top 30 OTUs in the colonic digesta of pigs.

|  |  |  |  |
| --- | --- | --- | --- |
| OTU ID | Relative abundance, % | Phylum | Genus |
| OTU1 |  13.26 | Firmicutes | *Streptococcus* |
| OTU2 | 9.71 | Firmicutes | *Lactobacillus* |
| OTU3 | 8.29 | Proteobacteria | *Escherichia-Shigella* |
| OTU4 | 6.90 | Firmicutes | *Terrisporobacter* |
| OTU19 | 5.47 | Firmicutes | *Lactobacillus* |
| OTU8 | 5.06 | Firmicutes | *Clostridium sensu stricto 1* |
| OTU7 | 3.93 | Firmicutes | *Megasphaera* |
| OTU28 | 3.65 | Firmicutes | *Phascolarctobacterium* |
| OTU5 | 3.48 | Firmicutes | *[Ruminococcus] torques group* |
| OTU39 | 3.35 | Bacteroidetes | *Prevotella 9* |
| OTU25 | 3.34 | Firmicutes | *Lachnospiraceae\_Unclassified*  |
| OTU11 | 2.74 | Firmicutes | *Turicibacter* |
| OTU18 | 2.53 | Bacteroidetes | *Prevotella 1* |
| OTU9 | 2.26 | Firmicutes | *Lachnospiraceae\_Unclassified*  |
| OTU44 | 2.15 | Firmicutes | *Lactobacillus* |
| OTU13 | 2.02 | Firmicutes | *Lachnospiraceae NK4A136 group* |
| OTU23 | 2.00 | Bacteroidetes | *Alloprevotella* |
| OTU47 | 1.78 | Bacteroidetes | *Rikenellaceae RC9 gut group* |
| OTU32 | 1.76 | Bacteroidetes | *Bacteroidales S24-7 group\_Unclassified*  |
| OTU12 | 1.73 | Actinobacteria | *Bifidobacterium* |
| OTU20 | 1.68 | Firmicutes | *Ruminococcus 2* |
| OTU10 | 1.65 | Actinobacteria | *Collinsella* |
| OTU57 | 1.60 | Firmicutes | *Lachnospiraceae\_Unclassified*  |
| OTU30 | 1.58 | Bacteroidetes | *Parabacteroides* |
| OTU14 | 1.55 | Firmicutes | *Blautia* |
| OTU15 | 1.55 | Firmicutes | *Ruminococcaceae UCG-005* |
| OTU16 | 1.31 | Firmicutes | *Coprococcus 3* |
| OTU17 | 1.30 | Firmicutes | *[Eubacterium] coprostanoligenes group* |
| OTU6 | 1.29 | Firmicutes | *Subdoligranulum* |
| OTU250 | 1.10 | Firmicutes | *Subdoligranulum* |



**Fig. S1.** Alpha diversity analysis of colonic microbiota. Rarefaction curves (a) and rank-abundance curves (b) were calculated for reads exhibiting ≥97% sequence identity. CON: Control diet. LPA: Low-protein diets supplemented with free amino acids. LPC: Low-protein diets supplemented with casein hydrolysate.



**Fig. S2.** Heat-map of top 30 genera in the colonic digesta. A color gradient showed in the top-right expresses the relative abundance of colonic genera. CON: Control diet. LPA: Low-protein diets supplemented with free amino acids. LPC: Low-protein diets supplemented with casein hydrolysate.